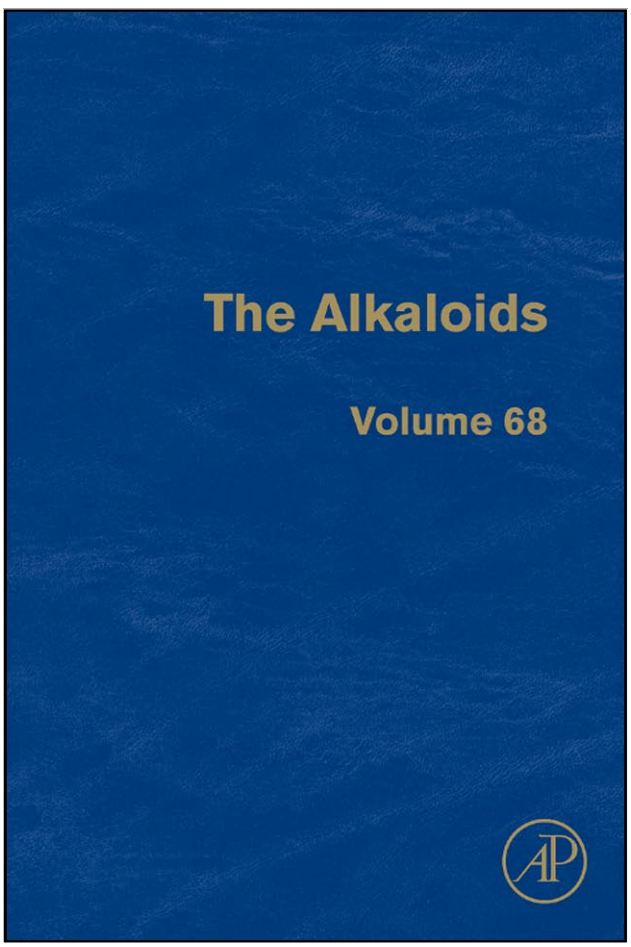

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CHAPTER 3

Alkaloids from the Genus *Duguetia*

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I. INTRODUCTION

Duguetia A. St.-Hil. (Annonaceae) is a genus of usually small, understory trees growing almost exclusively in the tropics of South America, with a small extension across the Panama Isthmus. It is now regarded as comprising close to 100 species, considering the recent inclusion of four African taxa, of which three were previously known as *Pachypodanthium* Engler & Diels. It is therefore one of the largest Annonaceous genera after *Guatteria* and *Annona*. Many studies have been conducted on the secondary metabolites present in different parts of *Duguetia* plants, from which essential oils, aromatic compounds, monoterpenes, diterpenes, triterpenes, flavonoids, and most typically alkaloids have been isolated and characterized. In common with the other ''primitive angiosperms,'' *Duguetia* species accumulate isoquinoline alkaloids, and more specifically 1-benzyl-1,2,3,4-tetrahydroisoquinolines, usually referred to simply as ''benzylisoquinolines,'' and their biosynthetic or biogenetically presumed derivatives. The literature reports studies on the alkaloids of about 16 *Duguetia* species (one of which was not clearly identified), resulting in the isolation and identification or characterization of 105 different alkaloids. Although many of these alkaloids are widely distributed, a few unusual groups of alkaloids appear to be specific to this genus.

II. BOTANICAL CONSIDERATIONS

The plants of the Annonaceae have traditionally been classed as part of the order Magnoliales. In the most recent consensus, the Magnoliales and Laurales constitute one of the two sister clades in the Magnoliidae, which are commonly regarded as the most ''primitive'' angiosperms in older classifications (1,2).

Regarding the occurrence of benzylisoquinoline alkaloids in the Annonaceae, other magnoliids, and more distantly related families, it is of interest to note that there is now good biochemical and molecular phylogenetic evidence for the evolution of benzylisoquinoline alkaloid biosynthesis in angiosperms from a common ancestor. Activity ascribable to the first enzyme in this biosynthetic tree, (*S*)-norcoclaurine synthase, occurs in 90 different plant species, and compares well with a molecular phylogeny. Phylogenetic analyses of norcoclaurine synthase, the berberine bridge enzyme, and several *O*-methyltransferases ''suggest a latent molecular fingerprint for benzylisoquinoline alkaloid biosynthesis in angiosperms not known to accumulate such alkaloids'' (3).

Duguetia was thought, on the basis of inflorescence and floral characters, to form an alliance with the very small neotropical genera *Duckeanthus*, *Fusaea*, and *Malmea*, and the African *Letestudoxa* (4). The monotypic *Pseudartabotrys* was later included and *Malmea* excluded (5), but incorporation of leaf, flower, fruit, and seed characters that had not been considered previously has led to a different grouping in which *Duguetia* (including *Pachypodanthium*) constitutes a clade of its own, close to a separate sister group including *Fusaea*, *Duckeanthus*, *Letestudoxa*, and *Pseudartabotrys* (6). Despite the inclusion of *Pachypodanthium* as ''African species of *Duguetia*,'' these plants still form a small, distinct cluster, perhaps not surprisingly together with *Duguetia riberensis* of Venezuela, in this cladistic analysis.

The genus has been further subdivided into 14 sections by Fries based on their morphological characters, but leaving some species in uncertain positions (7,8). These subdivisions have largely been upheld by a more recent study (9), and it is the system used in this review (Table I).

One third of all *Duguetia* species were analyzed in a study based on their genomic DNA sequences (41). That work supported the notion that *Duguetia*, like *Guatteria*, is monophyletic, with its most recent common ancestor dating back to 29.04 ± 4.52 million years ago (in the case of *Guatteria* this figure is 36.65 ± 2.50 mybp), although the authors concede that ''the accuracy of the absolute dates remains unassessed.'' A fossilized leaf from the middle Eocene period (about 38–48 mybp) from Western Tennessee, when the local climate was subtropical to tropical, has been classified as belonging to a *Duguetia* species (42), a conclusion that seems to conflict with the estimated DNA age of the genus. On the basis of its present geographic, trans-Atlantic distribution it was suggested that the *Duguetia* clade might predate the break-up of Gondwana (6). As the separation of Africa and South

America is believed to have been completed in the early Cretaceous (about 110 million years ago), and the age of the Annonaceae as a family is estimated to be as little as 82 million years (43), it seems necessary to assume long-distance dispersal over the widening early Atlantic Ocean, possibly across stepping-stones along the 80 million-year-old volcanic Sierra Leone Rise (to which the Ceará Rise should be added) (44) or, less likely, the more southerly Walvis Ridge (and Rio Grande Rise) (45). This hypothesis seems reasonable given the presence of Annonaceae in the Lesser Antilles, which would represent much more recent (Pliocene or even Pleistocene) events of a similar character (46).

III. ALKALOIDS FROM CHEMICALLY INVESTIGATED *DUGUETIA* SPECIES

The *Duguetia* species studied to date for their alkaloidal content are listed in Table I, ordered by sections, and in alphabetical order when appropriate. All of the alkaloids isolated from this genus have at least a formal isoquinoline-derived structure; including the 1-azaanthraquinone cleistopholine and the rare copyrine alkaloids, the 1-aza-7-oxoaporphines and 1-aza-4,5-dioxoaporphines. These alkaloids are classified as benzyltetrahydroisoquinolines, a single bisbenzyltetrahydroisoquinoline, berbines (tetrahydroprotoberberines), protoberberines, a morphinandienone, a proaporphine, and many aporphinoids and aporphinoid-related compounds. A large proportion of the aporphines are oxygenated at C7, a fairly common feature in the Annonaceae. 7-Methoxy derivatives are almost completely restricted to the African *Duguetia* species. Four *N*-formylnoraporphines have been identified. Three nitroso- or nitroaporphinoid derivatives isolated from *Duguetia furfuracea* might be artifacts, as discussed below. Several of the aporphinoids have the unusual 9,11-dioxygenation pattern in ring D which, aside from *Duguetia*, has only been found in one *Guatteria* species. As in *Guatteria*, some of the *Duguetia* aporphinoids bear a biogenetically intriguing carbon atom bonded to C7. Finally, a protoberberine–styrene adduct is a unique alkaloid from the African *Duguetia staudtii*. Table II lists the 105 alkaloids, including some possible artifacts, ordered according to their main structural features, as depicted in Figure 1 (Table III).

In many cases, the structures were known prior to their isolation from *Duguetia* species, or were very closely related to known alkaloids,

Table I Chemically investigated *Duguetia* species and their contained alkaloids

Section	Species	Alkaloid	Structure	Ref.(s)
Duguetia R. E. Fries	*D. furfuracea* (A. St.-Hil.) Benth. & Hook.	Reticuline	**1**	10
		Isochondodendrine	**3**	10
		Discretamine	**4**	10
		Isocorydine	**41**	10
		Norisocorydine	**40**	10
		Xylopine	**28**	10
		Obovanine	**30**	10
		Anonaine	**23**	10
		Asimilobine	**20**	10
		Atherospermidine	**86**	10
		Liriodenine	**83**	10
		Lanuginosine	**87**	10
		Duguetine	**76**	11
		N-Oxyduguetine	**77**	11
		Dicentrinone	**91**	11
		N-Methylglaucine	**36**	11
		N-Methyl-tetrahydropalmatine	**8**	11
		N-Nitrosoanonaine	**51**	12
		N-Nitrosoxylopine	**52**	12
		8-Nitroisocorydine	**42**	13
	D. odorata (Diels) J. F. Macbr.	Dehydrodiscretine	**16**	14
		Pseudopalmatine	**17**	14
		Oliveroline	**60**	14
		N-Methylguatterine	**66**	14

Table I *(Continued)*

Section	Species	Alkaloid	Structure	Ref.(s)
	D. stelechantha (Diels) R. E. Fries	Oxopukateine	**88**	15
		O-Methylmoschatoline	**85**	15
		Corypalmine	**5**	15
Hadrantha R. E. Fries	*D. hadrantha* (Diels) R. E. Fries	Hadranthine A	**99**	16
		Hadranthine B	**100**	16
		Imbiline-1	**101**	16
		Sampangine	**97**	16
		3-Methoxysampangine	**98**	16
Sphaerantha R. E. Fries	*D. calycina* Benoist	Discretamine	**4**	17
		10-Demethylxylopinine	**11**	17
		Xylopine	**28**	17
		Puterine	**31**	17
		O-Methylpukateine	**32**	17
		Obovanine	**30**	17
		Oxoputerine	**89**	17
		Atherosperminine	**94**	17
		Calycinine	**43**	17
		Noratherosperminine	**93**	18
		Duguecalyne	**54**	19
		N-Formylputerine	**53**	19
		Duguenaine	**47**	20
	D. obovata R. E. Fries	Xylopine	**28**	20
		Isolaureline	**29**	20

	N-Formylxylopine	**48**	20
	Buxifoline	**33**	20
	N-Methylbuxifoline	**34**	20
	N-Formylbuxifoline	**49**	20
	Anolobine	**27**	20
	Calycinine	**43**	20
	N-Methylcalycinine	**44**	20
	Duguevanine	**45**	20
	N-Formylduguevanine	**50**	20
	N-Methylduguevanine	**46**	20
	Oxobuxifoline	**90**	20
	Xylopinine	**12**	20
	Discretine	**10**	20
	(9*S*)-Sebiferine	**18**	20
D. spixiana Mart. (Colombia)	*N*-Oxycodamine	**2**	21,22
	N-Methylasimilobine	**21**	21
	Noroliveridine	**67**	21
	Oliveridine	**68**	21
	N-Oxyoliveridine	**70**	21
	Norpachyconfine	**56**	21
	Pachyconfine	**58**	21
	N-Oxypachyconfine	**59**	21
	Spixianine	**73**	21
	N-Oxyspixianine	**74**	21
	Duguexine	**71**	21
	N-Oxyduguexine	**72**	21

Table I *(Continued)*

Section	Species	Alkaloid	Structure	Ref.(s)
		Lanuginosine	**87**	21
		Atherosperminine	**94**	21
		N-Oxyatherosperminine	**95**	21
		Methoxyatherosperminine	**96**	21
		Spiduxine	**13**	21
		Duguespixine	**55**	21,23
	D. spixiana Mart. (Bolivia)	Anonaine	**23**	24
		Nornuciferine	**22**	24
		3-Hydroxynornuciferine	**25**	24
		O-Methylisopiline	**26**	24
		Noroliveridine	**67**	24
		Oliveridine	**68**	24
		N-Oxyoliveridine	**70**	24
		Duguexine	**71**	24
		Roemerolidine	**69**	24
		Nornuciferidine	**57**	24
		Rurrebanine	**63**	24
		Rurrebanidine	**62**	24
		Lysicamine	**84**	24
		Lanuginosine	**87**	24
		O-Methylmoschatoline	**85**	24
		Spiguetidine	**103**	24

		Spiguetine	**102**	24
		Xylopinine	**12**	24
		Tetrahydropalmatine	**7**	24
Calothrix R. E. Fries	*D. vallicola* J. F. Macbr.	*N*-Methyllaurotetanine	**37**	25
		Isocorydine	**41**	26
		Isoboldine	**38**	26
		Oliveridine	**68**	27
		Oliveroline	**60**	27
		Duguevalline	**92**	27
		O-Methylmoschatoline	**85**	27
		Xylopinine	**12**	26
		Discretine	**10**	26
		Pseudopalmatine	**17**	26
		Cleistopholine	**104**	27
		Glaziovine	**19**	26
Polyantha R. E. Fries	*D. eximia* Diels	*O*-Methylmoschatoline	**85**	28
		Oxopukateine	**88**	28
		Oxoputerine	**89**	28
Geanthemum R. E. Fries	*D. flagellaris* Huber	Nornuciferine	**22**	29,30
		Isopiline	**24**	29,30
		O-Methylisopiline	**26**	29,30
		Calycinine	**43**	29,30
		Duguevanine	**45**	29,30
		Pachypodanthine	**78**	29,30
		Oliveroline	**60**	29,30

Table I *(Continued)*

Section	Species	Alkaloid	Structure	Ref.(s)
		N-Oxyoliveroline	**61**	29,30
		Oliveridine	**68**	29,30
		Duguetine	**76**	29,30
Uncertain	*D. colombiana* Maas	*O*-Methylmoschatoline	**85**	31
	D. gardneriana Mart.	Discretamine	**4**	32
		Corypalmine	**5**	32
		Tetrahydropalmatine	**7**	32
	D. glabriuscula R. E. Fries	Polyalthine	**75**	33
		Oliveridine	**68**	33
		Oxobuxifoline	**90**	33
		Lanuginosine	**87**	33
	D. magnolioidea Maas	Discretamine	**4**	34
	D. trunciflora Maas	Reticuline	**1**	35
		Tetrahydropalmatine	**7**	35
		Corypalmine	**5**	35
		Discretamine	**4**	35
		Thaicanine	**14**	35
		Jatrorrhizine	**15**	35
Undetermined	*Duguetia* sp.	Norglaucine	**35**	36
		Dicentrine	**39**	36
		Duguetine	**76**	36
African species	*D. confinis*	Corypalmine	**5**	37
	(Engl. & Diels) Chatrou	Isocorypalmine	**6**	37

	Tetrahydropalmatine	**7**	37
	Govanine	**9**	38
	Discretine	**10**	38
	Oliveroline	**60**	37
	Guatterine	**64**	37
	N-Oxyguatterine	**65**	37
	Pachyconfine	**58**	37
	Pachypodanthine	**78**	38
	N-Acetylpachypodanthine	**80**	38
D. staudtii	Corypalmine	**5**	39
(Engl. & Diels) Chatrou	Isocorypalmine	**6**	40
	Discretine	**10**	39
	N-Methylpachypodanthine	**79**	39
	Pachystaudine	**82**	39
	Norpachystaudine	**81**	39
	Liriodenine	**83**	39,40
	Staudine	**105**	39,40
	Pachypodanthine	**78**	39,40

Table II Alkaloids isolated from *Duguetia* species

Alkaloid type and name	Structure	Molecular formula	MW	Species	Ref.(s)
Benzylisoquinolines					
(+)-Reticuline	**1**	$C_{19}H_{23}NO_4$	329	*D. furfuracea*	10
				D. trunciflora	35
cis-N-Oxycodamine	**2**	$C_{20}H_{25}NO_5$	359	*D. spixiana*[a]	21,22
Bisbenzylisoquinoline					
Isochondodendrine	**3**	$C_{36}H_{28}N_2O_6$	594	*D. furfuracea*	10
Berbines (Tetrahydroprotoberberines)					
(−)-Discretamine	**4**	$C_{19}H_{21}NO_4$	327	*D. calycina*	17
				D. gardneriana	32
				D. furfuracea	10
				D. trunciflora	35
				D. magnolioidea	34
(−)-Corypalmine (Tetrahydrojatrorrhizine)	**5**	$C_{20}H_{23}NO_4$	341	*D. gardneriana*	32
				D. stelechantha	15
				D. trunciflora	35
				D. staudtii	39
				D. confinis	37
(−)-Isocorypalmine	**6**	$C_{20}H_{23}NO_4$	341	*D. staudtii*	40
				D. confinis	37
(−)-Tetrahydropalmatine (Rotundine)	**7**	$C_{21}H_{25}NO_4$	355	*D. confinis*	37
				D. spixiana[b]	24
				D. gardneriana	32
				D. trunciflora	35

N-Methyltetrahydropalmatine	**8**	$C_{22}H_{28}NO_4$	370	*D. furfuracea*	11
(−)-Govanine	**9**	$C_{20}H_{23}NO_4$	341	*D. confinis*	38
(−)-Discretine	**10**	$C_{20}H_{23}NO_4$	341	*D. obovata*	20
				D. vallicola	26
				D. confinis	38
				D. staudtii	39
(−)-10-Demethylxylopinine	**11**	$C_{20}H_{23}NO_4$	341	*D. calycina*	17
(−)-Xylopinine	**12**	$C_{21}H_{25}NO_4$	355	*D. obovata*	20
				D. spixiana[b]	24
				D. vallicola	26
(−)-Spiduxine	**13**	$C_{21}H_{23}NO_5$	369	*D. spixiana*[a]	21
(−)-Thaicanine	**14**	$C_{21}H_{25}NO_5$	371	*D. trunciflora*	35
Protoberberines					
Jatrorrhizine	**15**	$C_{20}H_{20}NO_4$	338	*D. trunciflora*	35
Dehydrodiscretine	**16**	$C_{20}H_{20}NO_4$	338	*D. odorata*	14
Pseudopalmatine	**17**	$C_{21}H_{22}NO_4$	352	*D. odorata*	14
				D. vallicola	26
Morphinandienone					
(9*S*)-Sebiferine	**18**	$C_{20}H_{23}NO_4$	341	*D. obovata*	20
Proaporphine					
(−)-Glaziovine	**19**	$C_{18}H_{19}NO_3$	297	*D. vallicola*	26
Aporphines sensu stricto					
Asimilobine	**20**	$C_{17}H_{17}NO_2$	267	*D. furfuracea*	10
N-Methylasimilobine	**21**	$C_{18}H_{19}NO_2$	281	*D. spixiana*[a]	21

Table II *(Continued)*

Alkaloid type and name	Structure	Molecular formula	MW	Species	Ref.(s)
Nornuciferine	**22**	$C_{18}H_{19}NO_2$	281	*D. spixiana*[b]	24
				D. flagellaris	29,30
Anonaine	**23**	$C_{17}H_{15}NO_2$	265	*D. spixiana*[b]	24
				D. furfuracea	10
Isopiline	**24**	$C_{18}H_{19}NO_3$	297	*D. flagellaris*	29,30
3-Hydroxynornuciferine	**25**	$C_{18}H_{19}NO_3$	297	*D. spixiana*[b]	24
O-Methylisopiline	**26**	$C_{19}H_{21}NO_3$	311	*D. spixiana*[b]	24
				D. flagellaris	29,30
Anolobine	**27**	$C_{17}H_{15}NO_3$	281	*D. obovata*	20
Xylopine	**28**	$C_{18}H_{17}NO_3$	295	*D. calycina*	17
				D. obovata	20
				D. furfuracea	10
Isolaureline	**29**	$C_{19}H_{10}NO_3$	309	*D. obovata*	20
Obovanine	**30**	$C_{17}H_{15}NO_3$	281	*D. calycina*	17
				D. furfuracea	10
Puterine	**31**	$C_{18}H_{17}NO_3$	295	*D. calycina*	17
O-Methylpukateine	**32**	$C_{19}H_{19}NO_3$	309	*D. calycina*	17
Buxifoline	**33**	$C_{19}H_{19}NO_4$	325	*D. obovata*	20
N-Methylbuxifoline	**34**	$C_{20}H_{21}NO_4$	339	*D. obovata*	20
Norglaucine	**35**	$C_{20}H_{23}NO_4$	341	*Duguetia* sp.	36
N-Methylglaucine	**36**	$C_{22}H_{28}NO_4$	370	*D. furfuracea*	11
N-Methyllaurotetanine	**37**	$C_{20}H_{23}NO_4$	341	*D. vallicola*	25
Isoboldine	**38**	$C_{19}H_{21}NO_4$	327	*D. vallicola*	26

Dicentrine	**39**	$C_{20}H_{21}NO_4$	339	*Duguetia* sp.	36
Norisocorydine	**40**	$C_{19}H_{21}NO_4$	327	*D. furfuracea*	10
Isocorydine	**41**	$C_{20}H_{23}NO_4$	341	*D. furfuracea*	10
				D. vallicola	26
8-Nitroisocorydine	**42**	$C_{20}H_{22}N_2O_6$	386	*D. furfuracea*	13
Calycinine	**43**	$C_{18}H_{17}NO_4$	311	*D. calycina*	17
				D. flagellaris	29,30
				D. obovata	20
N-Methylcalycinine	**44**	$C_{19}H_{19}NO_4$	325	*D. obovata*	20
Duguevanine	**45**	$C_{19}H_{19}NO_5$	341	*D. obovata*	20
				D. flagellaris	29,30
N-Methylduguevanine	**46**	$C_{20}H_{21}NO_5$	355	*D. obovata*	20
N-Formylnoraporphines					
N-Formylputerine	**47**	$C_{19}H_{17}NO_4$	323	*D. calycina*	19
N-Formylxylopine	**48**	$C_{19}H_{17}NO_4$	323	*D. obovata*	20
N-Formylbuxifoline	**49**	$C_{20}H_{19}NO_5$	353	*D. obovata*	20
N-Formylduguevanine	**50**	$C_{20}H_{21}NO_6$	369	*D. obovata*	20
N-Nitrosonoraporphines					
N-Nitrosoanonaine	**51**	$C_{17}H_{14}N_2O_3$	294	*D. furfuracea*	12
N-Nitrosoxylopine	**52**	$C_{18}H_{16}N_2O_4$	324	*D. furfuracea*	12
7-Alkyl-substituted-6a,7-dehydroaporphines					
Duguenaine	**53**	$C_{19}H_{15}NO_3$	305	*D. calycina*	20
Duguecalyne	**54**	$C_{20}H_{17}NO_4$	335	*D. calycina*	19
Duguespixine	**55**	$C_{19}H_{17}NO_3$	307	*D. spixiana*[a]	21,23

Table II *(Continued)*

Alkaloid type and name	Structure	Molecular formula	MW	Species	Ref.(s)
7-Hydroxyaporphines					
Norpachyconfine	**56**	$C_{17}H_{17}NO_3$	283	*D. spixiana*[a]	21
Nornuciferidine	**57**	$C_{18}H_{19}NO_3$	297	*D. spixiana*[b]	24
Pachyconfine	**58**	$C_{18}H_{19}NO_3$	297	*D. confinis*	37
				D. spixiana[a]	21
N-oxypachyconfine	**59**	$C_{18}H_{19}NO_4$	313	*D. spixiana*[a]	21
Oliveroline	**60**	$C_{18}H_{17}NO_3$	295	*D. confinis*	37
				D. flagellaris	29,30
				D. vallicola	27
				D. odorata	14
N-Oxyoliveroline	**61**	$C_{18}H_{17}NO_4$	311	*D. flagellaris*	29,30
Rurrebanidine	**62**	$C_{18}H_{19}NO_4$	313	*D. spixiana*[b]	24
Rurrebanine	**63**	$C_{19}H_{21}NO_4$	327	*D. spixaina*[b]	24
Guatterine	**64**	$C_{19}H_{19}NO_4$	325	*D. confinis*	37
N-Oxyguatterine	**65**	$C_{18}H_{17}NO_5$	341	*D.confinis*	37
N-Methylguatterine	**66**	$C_{20}H_{22}NO_4$	340	*D. odorata*	14
Noroliveridine	**67**	$C_{17}H_{15}NO_3$	281	*D. spixiana*[a,b]	21,24
Oliveridine	**68**	$C_{19}H_{19}NO_4$	325	*D. spixiana*[a,b]	21,24
				D. glabriuscula	33
				D. flagellaris	29,30
				D. vallicola	27
Roemerolidine	**69**	$C_{18}H_{17}NO_4$	311	*D. spixiana*[b]	24
N-Oxyoliveridine	**70**	$C_{19}H_{19}NO_5$	341	*D. spixiana*[a,b]	21,24

Duguexine	**71**	$C_{18}H_{17}NO_4$	311	*D. spixiana*[a,b]	21,24
N-Oxyduguexine	**72**	$C_{18}H_{17}NO_5$	327	*D. spixiana*[a]	21
Spixianine	**73**	$C_{19}H_{19}NO_5$	341	*D. spixiana*[a]	21
N-Oxyspixianine	**74**	$C_{19}H_{19}NO_6$	357	*D. spixiana*[a]	21
Polyalthine	**75**	$C_{20}H_{21}NO_5$	355	*D. glabriuscula*	33
Duguetine	**76**	$C_{20}H_{21}NO_5$	355	*Duguetia* sp.	36
				D. flagellaris	29,30
				D. furfuracea	11
N-Oxyduguetine	**77**	$C_{20}H_{21}NO_6$	371	*D. furfuracea*	11
7-Methoxyaporphines					
Pachypodanthine	**78**	$C_{18}H_{17}NO_3$	295	*D. staudtii*	39,47
				D. confinis	38
				D. flagellaris	29,30
N-Methylpachypodanthine	**79**	$C_{19}H_{19}NO_3$	309	*D. staudtii*	39
N-Acetylpachypodanthine	**80**	$C_{20}H_{19}NO_4$	337	*D. confinis*	38
7-Methoxy-4-hydroxyaporphines					
Norpachystaudine	**81**	$C_{18}H_{17}NO_4$	311	*D. staudtii*	39
Pachystaudine	**82**	$C_{19}H_{19}NO_4$	235	*D. staudtii*	39
Oxoaporphines					
Liriodenine	**83**	$C_{17}H_9NO_3$	275	*D. furfuracea*	10
				D. staudtii	39,40
Lysicamine	**84**	$C_{18}H_{13}NO_3$	291	*D. spixiana*[b]	24
O-Methylmoschatoline	**85**	$C_{19}H_{15}NO_4$	321	*D. spixiana*[b]	24
				D. stelechantha	15

Table II *(Continued)*

Alkaloid type and name	Structure	Molecular formula	MW	Species	Ref.(s)
				D. eximia	28
				D. vallicola	27
				D. colombiana	31
Atherospermidine	**86**	$C_{18}H_{11}NO_4$	305	*D. furfuracea*	10
Lanuginosine	**87**	$C_{18}H_{11}NO_4$	305	*D. glabriuscula*	33
				D. furfuracea	10
				D. spixiana[a,b]	21,24
Oxopukateine	**88**	$C_{17}H_9NO_4$	291	*D. eximia*	28
				D. stelechantha	15
Oxoputerine	**89**	$C_{18}H_{11}NO_4$	305	*D. eximia*	28
				D. calycina	17
Oxobuxifoline	**90**	$C_{19}H_{13}NO_5$	335	*D. obovata*	20
				D. glabriuscula	33
Dicentrinone	**91**	$C_{19}H_{13}NO_5$	335	*D. furfuracea*	11
Duguevalline	**92**	$C_{20}H_{15}NO_6$	365	*D. vallicola*	27
Aminoethylphenanthrenes (6,6a-Secoaporphines)					
Noratherosperminine	**93**	$C_{19}H_{21}NO_2$	295	*D. calycina*	18
Atherosperminine	**94**	$C_{20}H_{23}NO_2$	309	*D. spixiana*[a]	21
				D. calycina	17
N-Oxyatherosperminine	**95**	$C_{22}H_{23}NO_3$	325	*D. spixiana*[a]	21
Methoxyatherosperminine	**96**	$C_{21}H_{25}NO_3$	339	*D. spixiana*[a]	21

Copyrine alkaloids					
1-Aza-7-oxoaporphines					
Sampangine	**97**	$C_{15}H_8N_2O$	232	*D. hadrantha*	16
3-Methoxysampangine	**98**	$C_{16}H_{10}N_2O_2$	246	*D. hadrantha*	16
1-Aza-4,5-dioxoaporphines					
Hadranthine A	**99**	$C_{18}H_{14}N_2O_4$	322	*D. hadrantha*	16
Hadranthine B	**100**	$C_{16}H_{10}N_2O_3$	278	*D. hadrantha*	16
Imbiline-1	**101**	$C_{17}H_{12}N_2O_3$	292	*D. hadrantha*	16
Azahomoaporphines					
Spiguetine	**102**	$C_{18}H_{16}N_2O_3$	308	*D. spixiana*[b]	24
Spiguetidine	**103**	$C_{19}H_{18}N_2O_3$	322	*D. spixiana*[b]	24
Azaanthraquinone					
Cleistopholine	**104**	$C_{14}H_9NO_2$	223	*D. vallicola*	27
Protoberberine–styrene adduct					
Staudine	**105**	$C_{31}H_{33}NO_7$	531	*D. staudtii*	39,48

[a] *D. spixiana* from Colombia.
[b] *D. spixiana* from Bolivia.

Table III Alphabetical list of alkaloids isolated from the genus *Duguetia* with their synonyms and structure numbers

Name	Structure	Name	Structure
N-Acetylpachypodanthine	**80**	Imbiline-1	**101**
Anolobine	**27**	Isoboldine (*N*-Methyllaurelliptine)	**38**
Anonaine	**23**	Isochondodendrine	**3**
Asimilobine	**20**	Isocorydine (Artabotrine, Luteanine)	**41**
Atherospermidine (Psilopine)	**86**	(−)-Isocorypalmine	**6**
Atherosperminine	**94**	Isolaureline (*N*-Methylxylopine)	**29**
Buxifoline	**33**	Isopiline	**24**
Calycinine	**43**	Jatrorrhizine	**15**
Cleistopholine	**104**	Lanuginosine (Oxoxylopine)	**87**
(−)-Corypalmine (Tetrahydrojatrorrhizine)	**5**	Liriodenine (Oxoushinsunine, Micheline B, Spermatheridine)	**83**
Dehydrodiscretine	**16**	Lysicamine (Oxonuciferine)	**84**
(−)-10-Demethylxylopinine	**11**	Methoxyatherosperminine	**96**
Dicentrine (*N*,*O*-Dimethylactinodaphnine, Eximine)	**39**	3-Methoxysampangine	**98**
Dicentrinone	**91**	*N*-Methylasimilobine	**21**
(−)-Discretamine	**4**	*N*-Methylbuxifoline	**34**
(−)-Discretine	**10**	*N*-Methylcalycinine	**44**
Duguecalyne	**54**	*N*-Methylduguevanine	**46**
Duguenaine	**53**	*N*-Methylglaucine	**36**
Duguespixine	**55**	*N*-Methylguatterine	**66**
Duguetine	**76**	*O*-Methylisopiline (*O*-Methylnorlirinine)	**26**
Duguevalline	**92**	*N*-Methyllaurotetanine (Lauroscholtzine, Rogersine)	**37**

Table III *(Continued)*

Name	Structure	Name	Structure
N-Oxyoliveridine	**70**	Staudine	**105**
N-Oxyoliveroline	**61**	(−)-Tetrahydropalmatine (Rotundine)	**7**
N-Oxypachyconfine	**59**	(−)-Thaicanine	**14**
N-Oxyspixianine	**74**	Xylopine (*O*-Methylanolobine)	**28**
Pachyconfine	**58**	(−)-Xylopinine	**12**
Pachypodanthine	**78**		

and in other instances the structure elucidations were straightforward, relying largely on the NMR spectra of the alkaloids. For this reason, in this section only the more problematic structure assignments will be discussed.

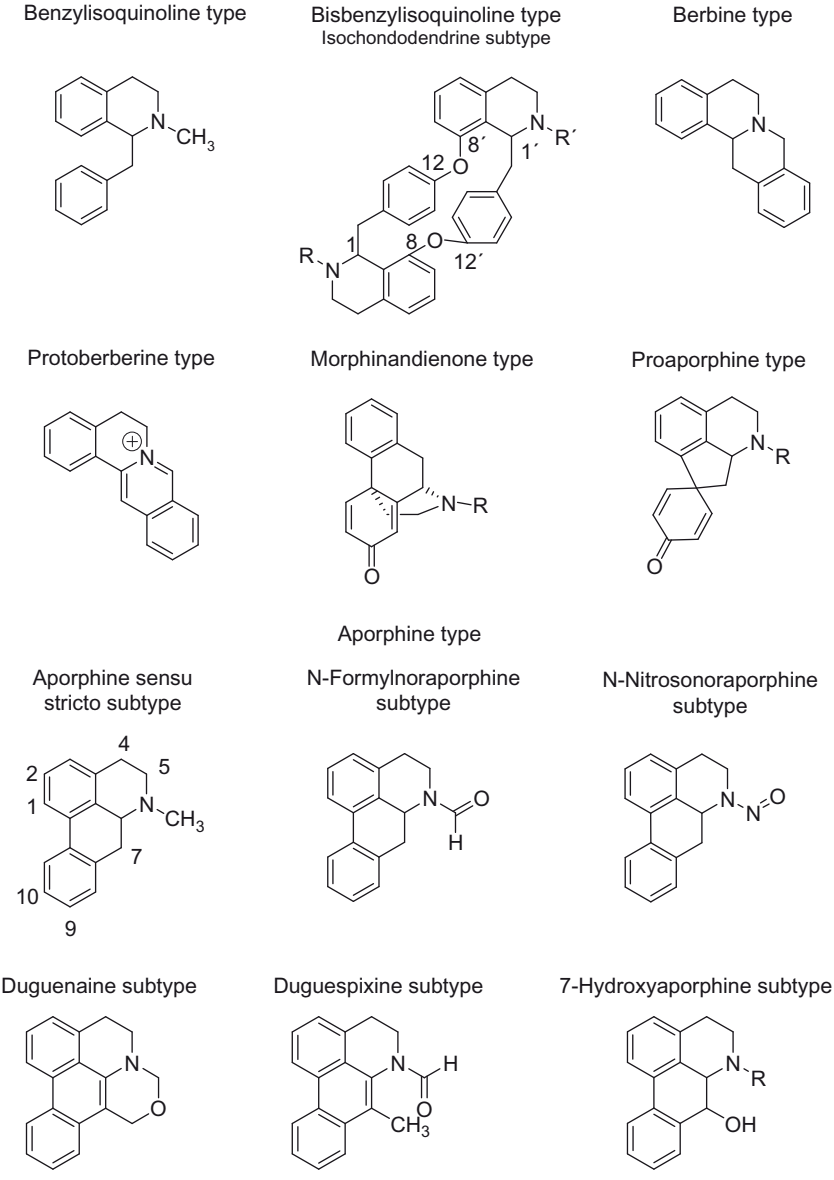


Figure 1 Structural types of alkaloids isolated from *Duguetia* species.

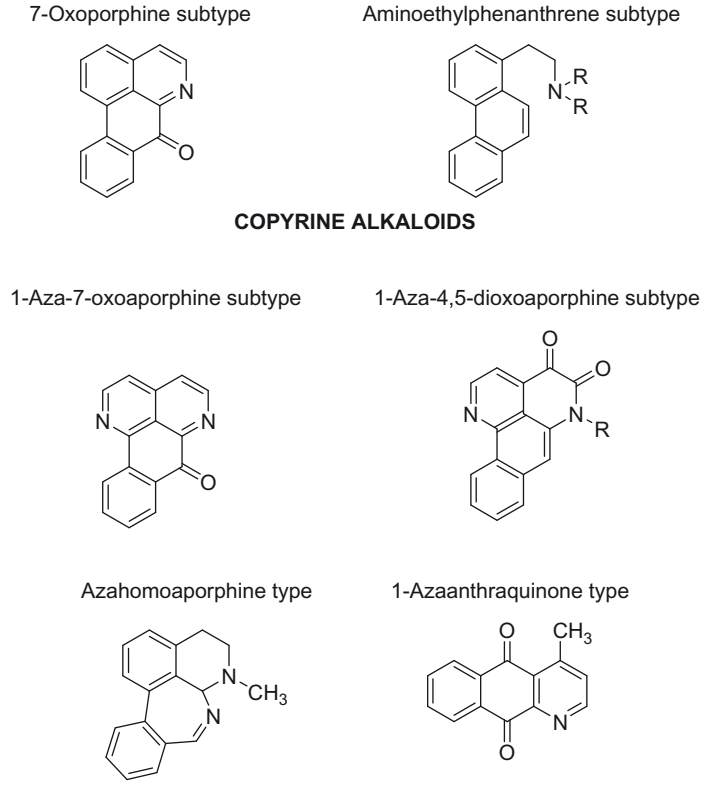
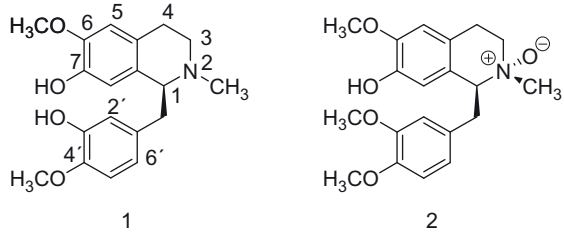


Figure 1 (*Continued*)

A. Benzyltetrahydroisoquinolines

Only two, unelaborated, benzyltetrahydroisoquinolines have been reported from the genus *Duguetia*, namely, reticuline (**1**), isolated from *Duguetia trunciflora* and *D. furfuracea* (10,35), and *cis-N*-oxycodamine (**2**), isolated from *Duguetia spixiana* (21,22).



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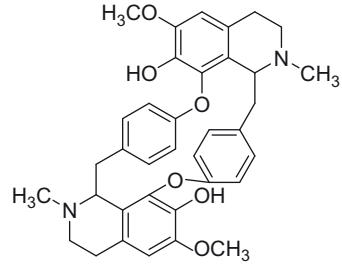
B. Bisbenzyltetrahydroisoquinolines

The head-to-tail/head-to-tail dimer isochondodendrine (**3**), isolated from *D. furfuracea* (10), is the only bisbenzyltetrahydroisoquinoline recorded to date from this genus.



3

C. Berbines and Protoberberines

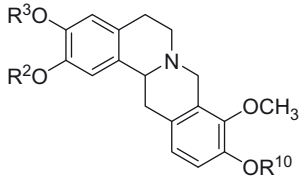
The berbines or tetrahydroprotoberberines appear to be widely distributed in the genus *Duguetia* (10 out of 15–16 species studied). Although quantitative analyses are lacking, it is noteworthy that these alkaloids comprise more than 50% of the mass of alkaloids isolated from the bark of the African *D. confinis*, and about 20% of *D. staudtii*, while they are apparently less abundant in the New World species. It is also noteworthy that, aside from their common precursor reticuline (**1**), the other five alkaloids isolated from *D. trunciflora* are members of this structural type, as do all three *Duguetia gardneriana* alkaloids. With the exception of spiduxine (**13**, only known so far from *D. spixiana*) and thaicanine (**14**, from *D. trunciflora*, but isolated previously from other, non-Annonaceous species), their structures are quite commonplace.

A single paper on the constituents of *D. trunciflora* reported the presence of reticuline (**1**), the berbines tetrahydropalmatine (THP) (rotundine) (**7**), tetrahydrojatrorrhizine (corypalmine) (**5**), discretamine (**4**), and thaicanine (**14**), and the protoberberine jatrorrhizine (**15**) (29). Although the optical rotations of the chiral members of this series were not published, all four berbines can be expected to have the usual *S*-configuration, and the same is true for the reticuline (**1**) isolated from this plant, if it is the biosynthetic precursor of the other isolates, and not, in this case, a dead-end metabolite with the *R* stereochemistry.

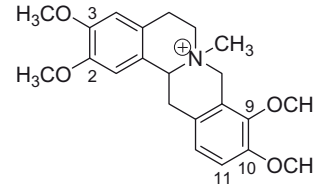
A report on the hypotensive and vasorelaxant effects of discretamine (**4**) from *Duguetia magnolioidea* Maas (34) refers to experimental details of the isolation ''according to the method described by Fechine *et al*. (2002)'' (35). Unfortunately, the report provides no information as to the location

where the plant was collected, its identification, or the existence of a voucher specimen.

In this genus, the quaternary *N*-methyltetrahydropalmatine (**8**) has only been isolated from *D. furfuracea*. Although its putative precursor, THP (also named rotundine, **7**) has not been reported from this species, its 3,10-dihydroxy analog discretamine (**4**) is present in *D. furfuracea*, *D. calycina*, *D. gardneriana*, *D. magnolioidea*, and *D. trunciflora* (10,17,32,34,35).



4 : $R^2 = CH_3$, $R^3 = R^{10} = H$
5 : $R^2 = R^{10} = CH_3$, $R^3 = H$
6 : $R^2 = H$, $R^3 = R^{10} = CH_3$
7 : $R^2 = R^3 = R^{10} = CH_3$



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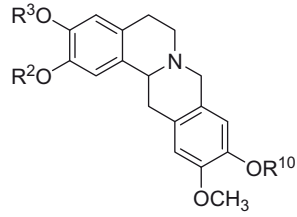
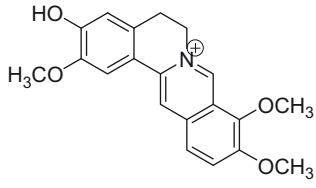


9 : $R^2 = H$, $R^3 = R^{10} = CH_3$
10 : $R^2 = R^{10} = CH_3$, $R^3 = H$
11 : $R^2 = R^3 = CH_3$, $R^{10} = H$
12 : $R^2 = R^3 = R^{10} = CH_3$

Protoberberines, easily formed nonenzymatically on prolonged exposure of berbines to air, have been isolated less often from *Duguetia*, but the co-occurrence of jatrorrhizine (**15**) and its tetrahydro analog corypalmine (**5**=tetrahydrojatrorrhizine) in *D. trunciflora*, and of pseudopalmatine (**17**) and the corresponding xylopinine (**12**) in *Duguetia vallicola* suggest that at least in these species they might be artifacts of storage or isolation. Thaicanine (**14**) is presumably a hydroxylation metabolite of THP (**7**). The C12-formylated spiduxine (**13**) from

Colombian *D. spixiana* is viewed as a (tetrahydro)retroprotoberberine (see Section V).



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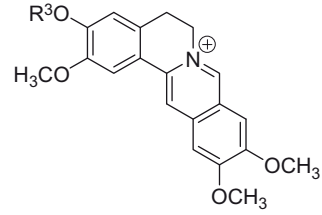
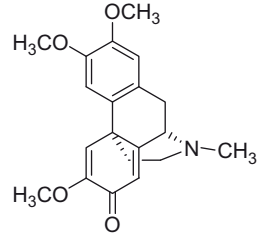


16 : $R^3 = H$
17 : $R^3 = CH_3$

D. Morphinandienone

(9*S*)-Sebiferine (**18**) is the only morphinandienone reported from this genus, as a constituent of *Duguetia obovata* (20).

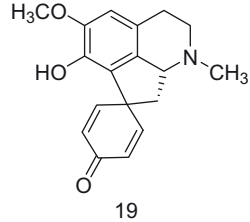


18

E. Aporphinoids

1. Proaporphines

Proaporphines, like the morphinandienones, seem to be uncommon in *Duguetia*. Only glaziovine (**19**) has been reported from the leaves of *D. vallicola* in which it is quite abundant (26).



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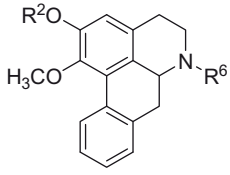
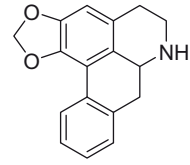
2. Aporphines *sensu stricto*

Aporphinoids in general are richly represented in the genus *Duguetia*. Aporphines *sensu stricto* **43**–**46**, *N*-formylduguevanine (**50**), the 7-hydroxyaporphines (**73**–**74**), and the oxoaporphine duguevalline (**92**), present the unusual 9,11-dioxygenation pattern.



20 : $R^2 = R^6 = H$
21 : $R^2 = H$, $R^6 = CH_3$
22 : $R^2 = R^6 = CH_3$



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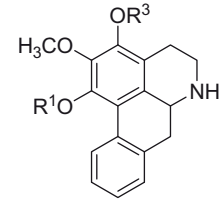
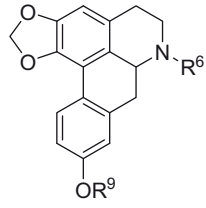
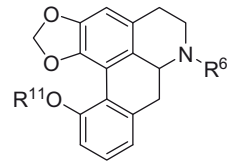


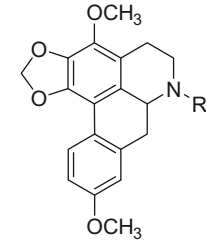
24 : $R^1 = H$, $R^3 = CH_3$
25 : $R^1 = CH_3$, $R^3 = H$
26 : $R^1 = R^3 = CH_3$



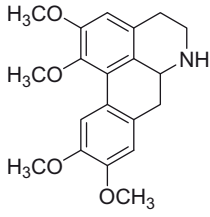
27 : $R^6 = R^9 = H$
28 : $R^6 = H$, $R^9 = CH_3$
29 : $R^6 = R^9 = CH_3$



30 : $R^6 = R^{11} = H$
31 : $R^6 = H$, $R^{11} = H$
32 : $R^6 = H$, $R^{11} = CH_3$



33 : $R^6 =$ H,
34 : $R^6 = CH_3$



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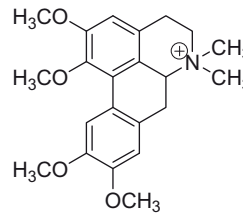

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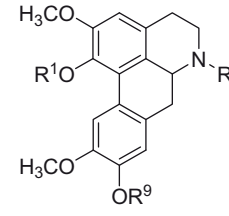
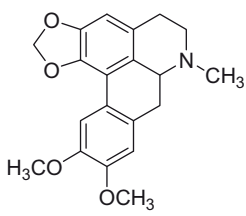


37 : $R^1 = R^6 = CH_3$, $R^9 = H$
38 : $R^1 = R^9 = H$, $R^6 = CH_3$



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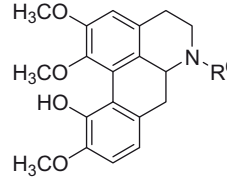
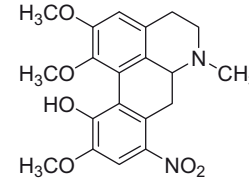


40 : R^6 = H
41 : R^6 = CH_3



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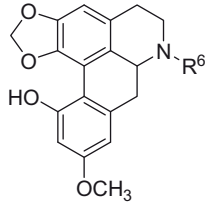
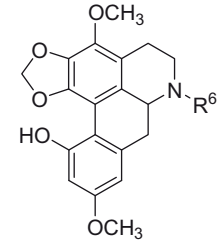


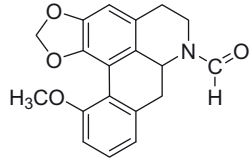
43 : R^6 = H
44 : R^6 = CH_3



45 : R^6 = H
46 : R^6 = CH_3

3. *N*-Formylaporphines

Four *N*-formylaporphines have been reported from the genus *Duguetia*, namely, *N*-formylputerine (**47**) from *D. calycina* (19), and *N*-formylxylopine (**48**), *N*-formylbuxifoline (**49**), and *N*-formylduguevanine (**50**) from *D. obovata* (20).



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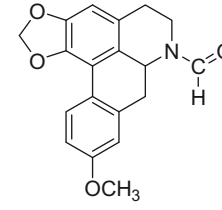

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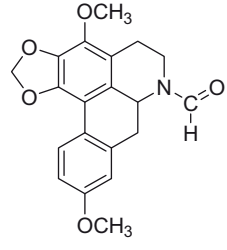

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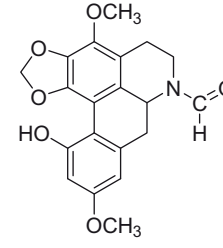
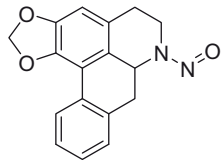


50

4. *N*-Nitrosoaporphines

Two *N*-nitrosonoraporphines, *N*-nitrosoanonaine (**51**) and *N*-nitrosoxylopine (**52**) have been reported from *D. furfuracea*. The structure of *N*-nitrosoanonaine (**51**) was confirmed by X-ray crystallography (12). The same authors have very recently reported the presence of 8-nitroisocorydine (**42**) in the same plant (13).



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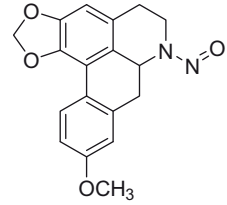
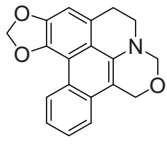


52

5. 7-Alkyl-6*a*,7-didehydroaporphines

Duguenaine (**53**) and duguecalyne (**54**) were isolated from *D. calycina* (19,20), and duguespixine (**55**) from the bark of the Colombian *D. spixiana* (21,23). The latter alkaloid was also found in *Guatteria sagotiana* (49), but to date duguecalyne (**54**) and duguenaine (**53**) seem to be exclusively *Duguetia* metabolites.



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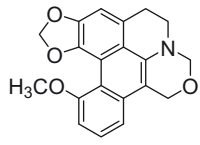

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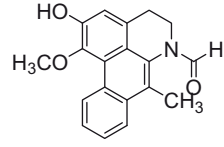
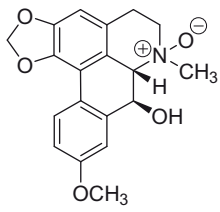


55

6. 7-Hydroxyaporphines

The genus *Duguetia* is remarkably rich in 7-hydroxylated aporphines, of which a small number have also been isolated from *Guatteria* species. Although only found in one half of the species studied, they account for nearly two thirds of the mass of alkaloids isolated from both Colombian and Bolivian *D. spixiana*.



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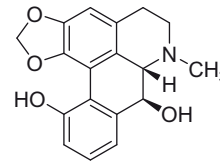

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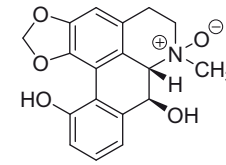

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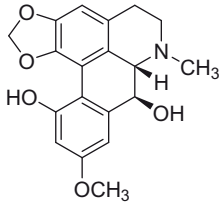

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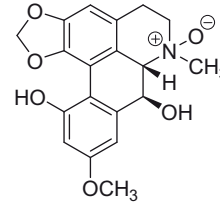

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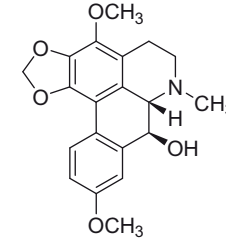

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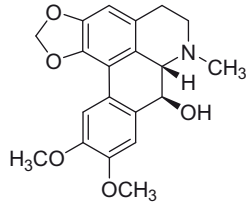

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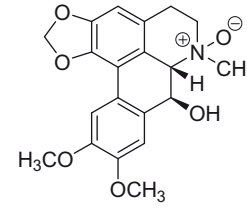
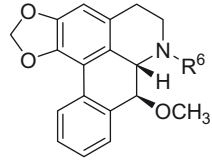
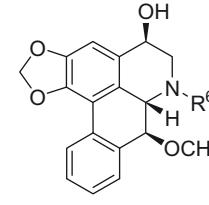


77

The closely related pachypodanthine (**78**), *N*-methylpachypodanthine (**79**), *N*-acetylpachypodanthine (**80**), pachystaudine (**82**), and norpachystaudine (**81**), all C7 methoxylated, are characteristic of the African species *D. staudtii* and *D. confinis* (formerly designated as *Pachypodanthium*). Although a few other C4–C7 oxygenated aporphines (e.g., stephadiolamine) and oxoaporphines are known, pachystaudine (**82**) and its nor-analog **81** seem to be the only aporphinoids characterized to date with both C4 hydroxy and C7 methoxy substituents.



78: $R^6 = H$
79: $R^6 = CH_3$
80: $R^6 = COCH_3$

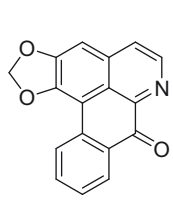


81: $R^6 = H$
82: $R^6 = CH_3$

7. Oxoaporphines

Nine 7-oxoaporphine alkaloids (7-oxo-4,5,6,6*a*-tetradehydroaporphines) have been isolated from *Duguetia* species, scattered throughout the genus. Perhaps significantly, all three alkaloids identified as constituents of *Duguetia eximia* belong in this group (28).

So far, duguevalline (**92**) is only the second oxoaporphine known to have the unusual 9,11-dioxygenation pattern. The other, oxoisocalycinine, was isolated from *Guatteria discolor* (50).



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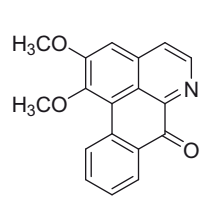

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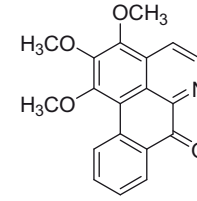

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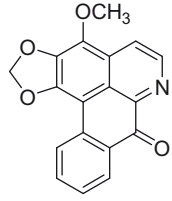

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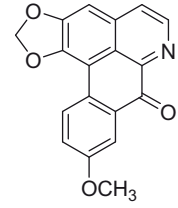

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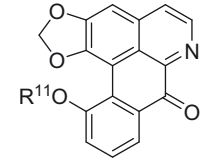
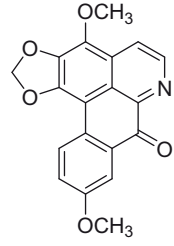


88 : R^{11} = H
89 : R^{11} = CH_3



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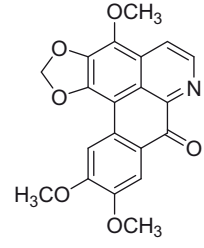

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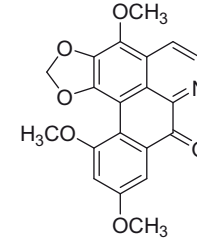

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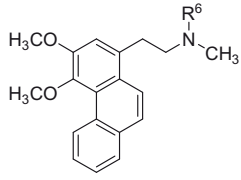
F. Miscellaneous Aporphinoid- and Berbinoid-Related Alkaloids

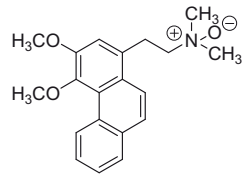
1. Aminoethylphenanthrenes

Four 1-aminoethylphenanthrenes, or 6,6*a*-secoaporphines, have been isolated from *Duguetia* species, these are: atherosperminine (**94**, from

D. spixiana and *D. calycina*), its *N*-oxide (**95**, from *D. spixiana*), noratherosperminine (**93**, from *D. calycina*), and methoxyatherosperminine (**96**, from *D. spixiana*).



93 : R^6 = H
94 : R^6 = CH_3



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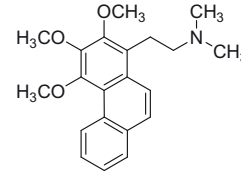
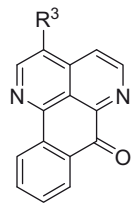


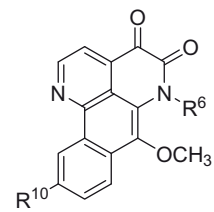
96

2. Copyrine Alkaloids

The relatively rare 1-azaaporphinoids are often referred to as copyrine alkaloids, by analogy with the term isoquinoline alkaloids, as copyrine is the trivial name of the 2,7-diazanaphthalene nucleus. Three 1-aza-4,5-dioxo-7-methoxy-6*a*,7-didehydroaporphines and two 1-aza-7-oxo-4,5,6,6*a*-tetradehydroaporphines were isolated from *Duguetia hadrantha* (16). The fact that these five unusual compounds are the only alkaloids isolated from this particular species, and that they have been found in no other *Duguetia* species, is probably a consequence of the antimalarial/antifungal bioassay-guided fractionation of the plant extract. They are biogenetically related to cleistopholine (**104**), which in this genus has only been recorded as a constituent in *D. vallicola*, and to other annonaceous 1-azaanthra-9,10-quinone derivatives with scattered occurrence in the genera *Annona*, *Cleistopholis*, *Guatteria*, *Meiogyne*, *Porcelia*, *Hornschuchia*, and *Cananga* (51–56).



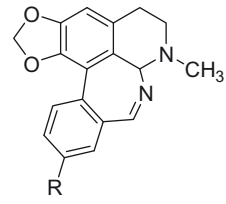
97 : R^3 = H
98 : R^3 = OCH_3



99 : R^6 = CH_3, R^{10} = OCH_3
100 : R^6 = R^{10} = H
101 : R^6 = CH_3, R^{10} = H

3. Azahomoaporphines

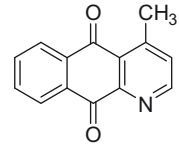
The only two azahomoaporphines found in the genus *Duguetia* are spiguetine (**102**) and spiguetidine (**103**), reported exclusively from a Bolivian accession of *D. spixiana*. They were not isolated from plant material collected in Colombia (24). They are members of a rare alkaloid structural type found only in this species, in *G. sagotiana* (dragabine), and in *Meiogyne virgata* (nordragabine), all in the family Annonaceae.



102 : R = OCH_3
103 : R = OH

4. Azaanthraquinone

Cleistopholine (**104**), the prototype of the few natural 1-aza-9,10-anthracenedione alkaloids known to date, was isolated from *D. vallicola* (27), and has also been found in several other Annonaceous genera.



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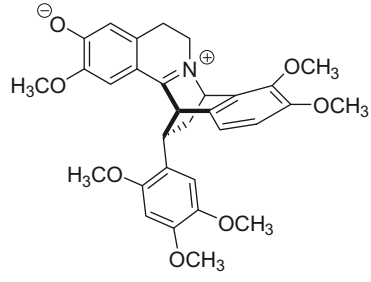
5. Protoberberine–Styrene Adduct

The structurally unique staudine (**105**) has only been isolated from *D. staudtii* (39,48).



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IV. STRUCTURE AND CHEMISTRY

A. Benzyltetrahydroisoquinolines

Although the configuration of the reticuline (**1**) isolated from *D. trunciflora* was not reported, it seems likely that it is the *S* isomer, as in *D. furfuracea*, and therefore is the immediate precursor of (*S*)-codamine and its *N*-oxide (**2**). The small amount of **2** isolated did not allow its absolute configuration to be determined, but it is depicted here as the more likely (*S*)-reticuline-derived *S* isomer (although in the original reference it is shown with the *R* configuration). The berbines and the 1,2,9,10- and 1,2,10,11-dioxygenated aporphines, of which there are a few in the source plant of *cis*-*N*-oxycodamine, the Colombian accession of *D. spixiana*, are generally derived from (*S*)-reticuline (**1**).

B. Bisbenzyltetrahydroisoquinoline

The complete assignments of the ^{1}H NMR and ^{13}C NMR spectra of isochondodendrine (**3**) have been published (10).

C. Berbinoids

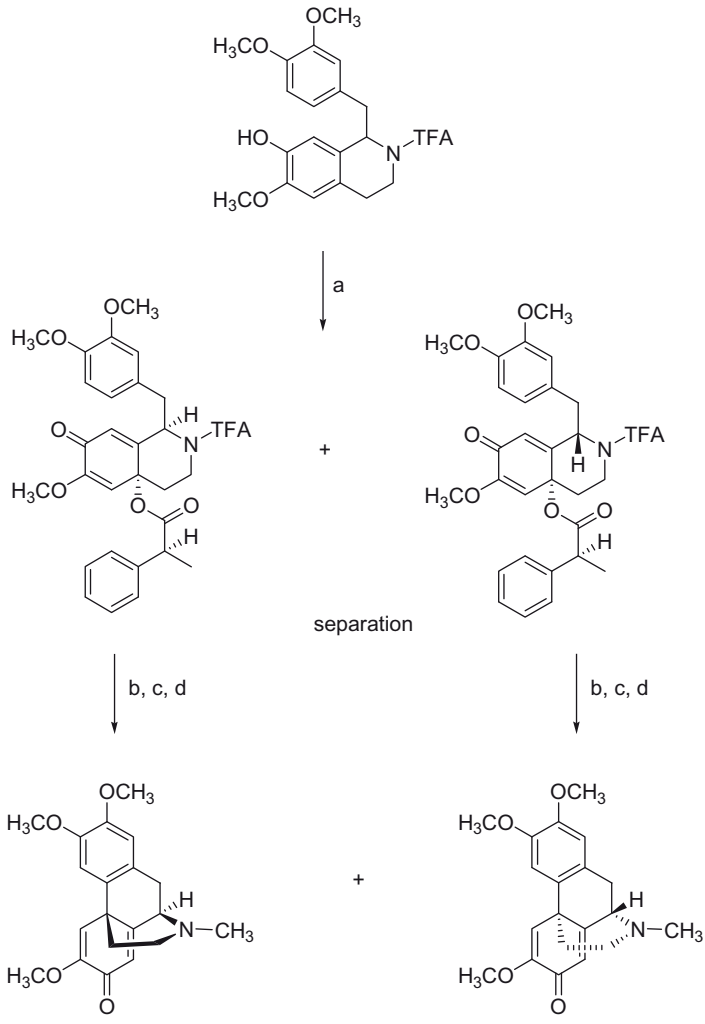
Quite surprisingly, the presence of (*R*)-dicentrine (**39**) and its 7-hydroxy derivative duguetine (**76**) was reported in an unidentified *Duguetia* species (36). This configuration flies in the face of biogenetic theory, but seems to be supported by the negative optical rotation of both alkaloids at 589 nm, and the ORD spectrum of the latter alkaloid. Unfortunately, the only recent report on the reisolation of duguetine from *Duguetia flagellaris* gives no details of its identification or of its physical (including optical rotation) and spectral properties (29,30).

D. Morphinandienone

The stereochemistry of (9*S*)-sebiferine (**18**), which is opposite to that of the morphine alkaloids of *Papaver* species, was demonstrated on the basis of the crystal structure determination of its methiodide (57). Both (9*S*)-sebiferine (**18**) and its enantiomer have been synthesized via *p*-quinol esters starting from the diastereomeric products of the lead tetraacetate oxidation of racemic *N*-trifluoroacetylnorcodamine in (*S*)-2-phenylpropionic acid (58) (Scheme 1).

E. Aporphinoids

The *N*-nitroso, non-phenolic noraporphines **51** and **52** were isolated from a 95% ethanolic extract of the leaves of *D. furfuracea* which was treated with 3% HCl. The authors appropriately state that *N*-nitrosamines can be



Scheme 1 Reagents and conditions: a. $Pb(OAc)_4$, (*S*)-2-phenylpropionic acid; b. TFA, CH_3CN, $-30^{\circ}C$; c. *N*-deprotection; d. *N*-methylation.

carcinogenic and/or mutagenic (59), and also remark that they ''can be regarded as potential NO/NO^+ donors, thus playing an important role in the regulation of many physiological functions'' (60). However, they do not address the possibility that these *N*-nitroso-alkaloids are artifacts of the isolation process.

Nitrates and nitrites commonly accumulate in higher plants. Their occurrence in dietary vegetables has been viewed since at least 1964 as a health hazard (61), and has been the subject of numerous subsequent

publications. Moreover, treatment of some secondary amine alkaloids with nitric acid has been known to lead to the formation of *N*-nitroso derivatives since the end of the 19th century (62), and the *N*-nitrosation of secondary amines occurs readily with inorganic nitrites and acid. It therefore seems possible that the *N*-nitrosoanonaine (**51**) and *N*-nitrosoxylopine (**52**) isolated by Carollo *et al*. were formed on acidification of the ethanol extract of the plant. What concentration of nitrate or nitrite was present in the *Duguetia* sample studied by these authors is a question that would seem to be worth addressing.

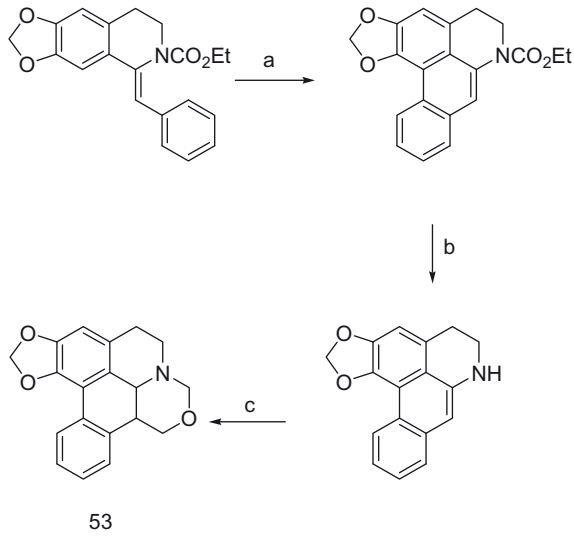
In the opinion of the authors, nitration of isocorydine at the free C8 position, *para* to a phenol function to give 8-nitroisocorydine (**42**), should occur under very mild conditions. This reinforces the hypothesis that these unusual alkaloids are formed either in the living plant or during the extraction procedure by (presumably nonenzymatic) reaction with nitrates or nitrites present in the plant material. The 8-nitroisocorydine structure, however, does not seem to have been established unambiguously. The *N*-methyl ^{1}H resonance is not reported (its ^{13}C resonates at the normal chemical shift value of 43.9 ppm), and the mass-spectral fragmentation shows a possibly suspicious loss of NO from the molecular ion. Is it possible that this isolate is 8-nitrosoisocorydine *N*-oxide, with one or two apparently anomalous *N*-methyl resonances as described by Debourges *et al*. (22). It is probably important to remember that *D. furfuracea* is one of the three *Duguetia* species known to accumulate at least one aporphine *N*-oxide (11).

A biomimetic synthesis of the unusual oxazine-condensed aporphine duguenaine (**53**) and some related analogs has been reported, based on the UV irradiation of an ethanol-tetrahydrofuran solution of 1-benzylidene-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline-2-ethoxycarboxylate in the presence of iodine to produce *N*-ethoxycarbonyldehydro-anonaine. This was followed by *N*-deprotection under basic conditions and quenching with aqueous citric acid to yield the dehydroanonaine salt. The oxazine ring was introduced by treating dehydroanonaine with aqueous formaldehyde at room temperature for 24 h (Scheme 2) (63).

An alternative synthesis of duguenaine (**53**) was published almost simultaneously, using anonaine (**23**) as the starting material. Anonaine was treated with *N*-chlorosuccinimide yielding the corresponding *N*-chloroanonaine. Sodium ethoxide was added to the mixture and the resulting dehydroanonaine was treated with aqueous formaldehyde under reflux for 30 min to furnish **53** (Scheme 3) (23).

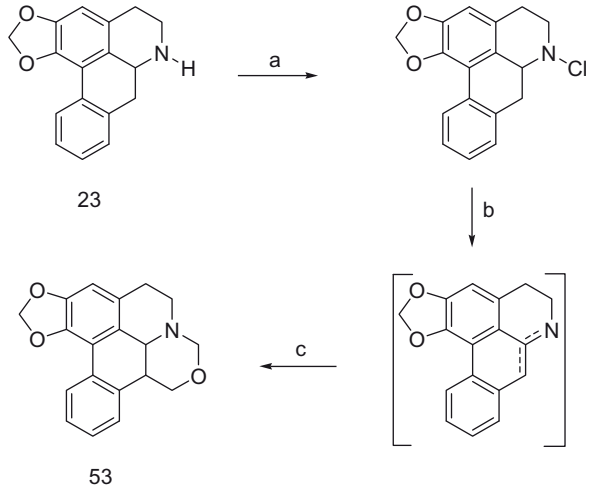
F. Miscellaneous Aporphinoid- and Berbinoid-Related Alkaloids

Imbiline 1 (**101**) has been synthesized fairly recently, in seven steps, starting from 4-methoxy-1-naphthylamine, in 9% overall yield (64).



53

Scheme 2 Reagents and conditions: a. UV, EtOH-THF, I_2, 9.5 h; b. KOH, EtOH, reflux 18 h; c. HCHO, dioxane, rt, 24 h.



23

53

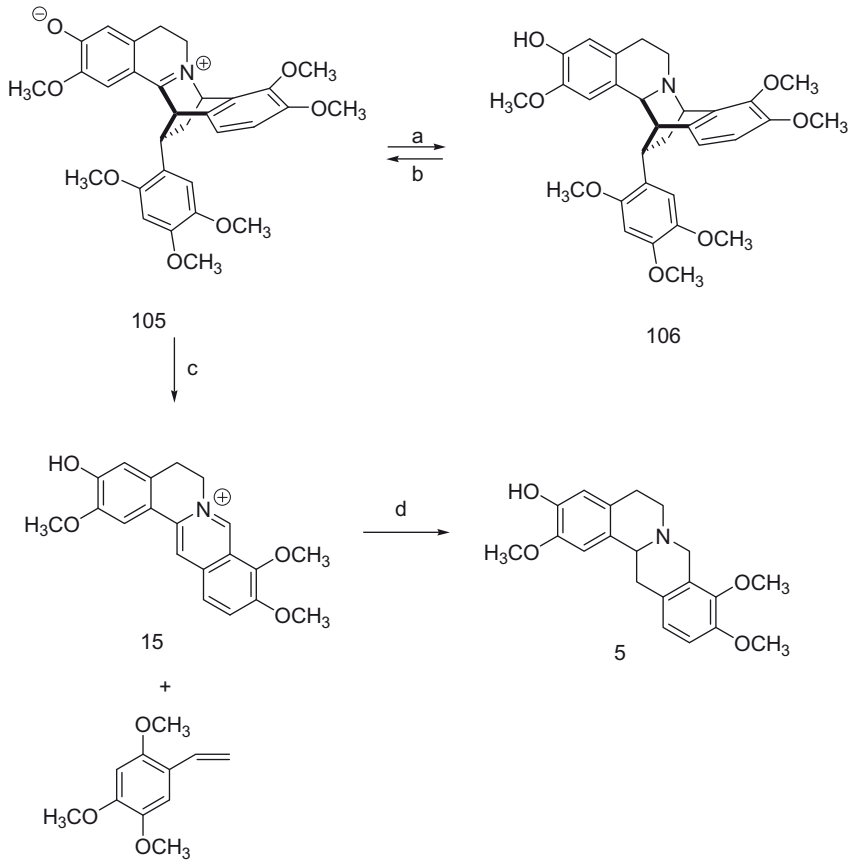
Scheme 3 Reagents and conditions: a. NCS; b. NaOEt; c. HCHO, reflux 0.5 h.

Staudine (**105**, relative configuration shown), isolated from *D. staudtii*, is a unique reverse electron demand Diels–Alder adduct of jatrorrhizine (**15**) and 2,4,5-trimethoxystyrene, which is an abundant metabolite in this plant. Its zwitterionic, rather than phenolic, character, suggested by its high melting point (205–206°C), was revealed by the absence of any change in its UV-VIS spectrum in alkaline solution, and by the failure of

an attempted acetylation with acetic anhydride in pyridine in the presence of 4-dimethylaminopyridine. The presence of a $C{=}N^+$ double bond was apparent from its IR spectrum, which exhibited a strong band at 1605 cm^{-1}. This band disappeared on reduction of staudine (**105**) with sodium borohydride in methanol to afford a dihydro derivative **106** that undergoes facile reoxidation to staudine (**105**) in the presence of air.

The ^1H NMR spectrum of staudine (**105**) showed the presence of six methoxy groups, two single proton multiplets at δ 4.45 and 5.17, and five aromatic proton singlets (one due to two protons, the others to one each). One of the methoxyl resonances (at 3.37 ppm) and one of the aromatic proton signals (at 5.32 ppm) exhibited unusual deshieldings which could be attributed to a structure with closely superimposed aromatic rings. The mass spectrum showed a weak (1%) molecular ion peak, and more abundant fragments at $m/z < 360$. Of particular interest were three peaks at m/z 194 (90%), 179 (40%), and 151 (37%), corresponding to a trimethoxystyrene. The base peak occurred at m/z 337 (M^+-194) with another strong signal at m/z 352 (30%, M^+-179). These data suggested that staudine (**105**) contains a benzylisoquinoline moiety in addition to the trimethoxystyrene moiety, which seem to undergo a retro-Diels–Alder reaction in the mass spectrometer. The ^{13}C NMR spectrum showed all the signals expected for 2,4,5-trimethoxystyrene, with the exception of the ethylene carbon resonances, and all the signals expected for corypalmine (tetrahydrojatrorrhizine, **5**), except for the C14 resonance, plus additional resonances at 32.8, 34.0, and 176.3 ppm. All these data, and further tentative assignments of the sp^3 ^{13}C resonances, showed that the structure of staudine (**105**) incorporates a 2,4,5-trimethoxystyrene moiety bonded through its vinyl side chain to C8 and C13 of corypalmine, but with a C14N double bond. This was confirmed by the pyrolysis of staudine (**105**) under high vacuum at 180°C, which led to the sublimation of 2,4,5-trimethoxystyrene, leaving a highly polar residue. Sodium borohydride reduction of this residue afforded the previously characterized dihydrostaudine (**106**) and corypalmine (**5**) (Scheme 4).

Definitive proof of the structure was provided by an X-ray crystallographic analysis, which showed unambiguously that the benzylic carbon of the styrene residue is bonded to C13 of the corypalmine moiety, and that the more distal styrene carbon atom is bonded to C8. Heating jatrorrhizine (**15**) and 2,4,5-trimethoxystyrene in bromobenzene at 100°C for 10 h produced only a small amount of staudine (**105**), identified by TLC, leading the authors to conclude that this alkaloid is not an isolation artifact (48). Nevertheless, this conclusion is still arguable considering that the same authors reported an $[\alpha]_D{=}0$ for this alkaloid with three stereogenic carbon atoms and, as the crystal structure shows, a highly dissymmetric arrangement of the three benzene chromophores which



Scheme 4 Conditions: a. $NaBH_4$, MeOH; b. Air; c. 180°C, 0.01 Torr, 6 h; d. KBH_4, MeOH.

could be expected to result in a fairly high optical rotation. The crystal packing was not reported and it is therefore not possible to determine if the eight molecules in the unit cell have the same configuration, or if the crystal itself is racemic.

It may be pointed out that 2,4,5-trimethoxystyrene, which is quite toxic to brine shrimp, but only weakly cytotoxic, has been reported as the major bioactive constituent of *Duguetia panamensis* Standley (no studies have been published on the alkaloids of this species) (65), and is also present in *Duguetia colombiana* (31).

V. BIOSYNTHESIS, BIOGENESIS, AND CHEMOSYSTEMATICS

No biosynthetic work has been conducted specifically on plants belonging to the Annonaceae. However, earlier studies of tetrahydrobenzylisoquinoline

alkaloid biosynthesis can be generalized to the more widespread *Duguetia* alkaloids. Regarding biogenetic speculations, some of which have been summarized in an earlier chapter of this series (51), the situation is similar. Some recent developments, both experimental and hypothetical, are reviewed here.

(*S*)-Reticuline (**1**) and codamine *cis-N*-oxide or oxycodamine (**2**) lie near the base of the biosynthetic branch leading to most of the *Duguetia* alkaloids. As the 1,2,9,10- and 1,2,10,11-oxygenated aporphines and the berbines are all derived from (*S*)-reticuline (**1**), but not codamine, the *cis-N*-oxycodamine of *D. furfuracea* can be regarded as a terminal biosynthetic product.

(*S*)-Reticuline (**1**) is the biosynthetic precursor of all known berbines and the 9,10- and 10,11-dioxygenated aporphinoids, and, through the unstable 1,2-dehydroreticuline, is also the precursor of (*R*)-reticuline, the common precursor of most morphinandienone alkaloids. Reasoning biogenetically, (−)-dicentrine (**39**) should originate by direct C8−C6′ coupling of (*R*)-reticuline. It is therefore of interest to note that 1,2-dehydroreticuline synthase, the enzyme at the branching point that separates (*R*)- and (*S*)-reticuline metabolites, has been partially purified and shown to not require a redox cofactor, accepting both (*S*)-reticuline and (*S*)-norreticuline as substrates (66).

The occurrence of isochondodendrine (**3**) as the sole *Duguetia* bisbenzyltetrahydroisoquinoline parallels the limited occurrence of benzyltetrahydroisoquinoline dimers in *Guatteria*. In the largest genus in the Annonaceae, these alkaloids, although many in number, appear to be restricted to *G. boliviana*, *G. guianensis*, and *G. megalophylla* (51,67). *Guatteria gaumeri*, reported to contain a bisbenzylisoquinoline, is a misnomer for *Malmea gaumeri*, now viewed as a synonym of *Malmea depressa* (68). Moreover, cladistic analysis indicates that the split between the branches leading to *Malmea* (the short branch clade of the Annonaceae) and to *Duguetia* and *Guatteria* (the long-branch clade) must have occurred about 60 million years ago, 20 million years before the differentiation of the latter genera (46). Within the long-branch clade, the only other genera for which bisbenzylisoquinolines have been recorded are *Isolona*, *Uvaria*, and *Xylopia*. This suggests that the cytochrome P450 oxidases that presumably catalyze the intermolecular oxidative phenol couplings (two in succession in the case of isochondodendrine) of two coclaurine units (69) are poorly expressed in this group.

In the last few years, particularly important contributions have been made to the knowledge of the berberine bridge enzyme. This protein, incorporating a unique, bi-covalently attached FAD prosthetic group (70), catalyzes the conversion of (*S*)-reticuline to (*S*)-scoulerine by oxidation of the *N*-methyl group and coupling *ortho* to the phenol group of the benzyl ring (71,72). A mechanism has been proposed involving the

removal of hydride from the *N*-methyl group by the FAD cofactor, and concerted carbon−carbon coupling combined with base-catalyzed proton abstraction (73). The enzyme also oxidizes the berbine alkaloid scoulerine to the protoberberine dehydroscoulerine, resembling (*S*)-tetrahydroprotoberberine oxidase (STOX) and canadine oxidase in this regard (74). (*S*)-Tetrahydroprotoberberine oxidase converts (*S*)-tetrahydrocolumbamine to columbamine in the metabolic pathway leading to berberine, jatrorrhizine, and palmatine in *Berberis* species (75). Canadine oxidase catalyzes an alternative route in which formation of the dioxole ring precedes the dehydrogenation leading to berberine (76).

(*S*)-Reticuline (**1**) is not the exclusive berbine precursor. Berberine bridge enzyme of *Eschscholtzia californica*, heterologously expressed in insect cells, transforms other (*S*)- (but not *R*-configured) tetrahydrobenzylisoquinolines with a 2′-hydroxy group into (*S*)-berbines, apparently regardless of the substitution pattern on the benzene ring of the isoquinoline moiety of the precursor (77). In *Corydalis* and *Macleaya* cell cultures both (*S*)-reticuline and (*S*)-protosinomenine (the isomer of reticuline with the positions of the ring A hydroxy and methoxy groups interchanged), but not their enantiomers, undergo the analogous cyclization to (*S*)-scoulerine and tetrahydropalmatrubine (its methoxy derivative at C2) (78). On the other hand, when racemic laudanine (the 7-*O*-methyl ether of reticuline) was fed to the cells, both enantiomers of scoulerine and of the 10,11-dioxygenated berbine corytenchine were formed, in different enantiomeric ratios (78).

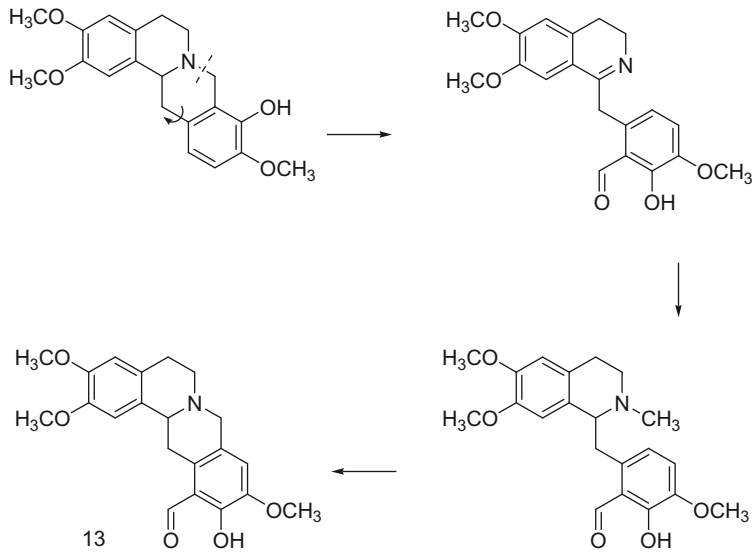
N-Methyltetrahydropalmatine (**8**) and the analogous *N*-methylstylopine and *N*-methylcanadine are synthesized in opium poppy from the corresponding racemic berbines by a recently cloned and characterized *S*-adenosyl-L-methionine:tetrahydroprotoberberine *cis*-*N*-methyltransferase (TNMT) which, however, does not modify (*S*)-scoulerine (79). The stereochemistry of the products was not determined. TNMT activity was detected in several other members of the Papaveraceae, but not in representatives of the Berberidaceae, Menispermaceae, and Ranunculaceae. It remains to be seen if this, or some similar, enzyme is active in the Annonaceae, and specifically in *D. furfuracea*.

It is worth pointing out that no 2-hydroxyberbines or protoberberines have been found in *Duguetia*, although there are a number of occurrences of 3-hydroxyberbines [discretamine (**4**), corypalmine (**5**), and discretine (**10**)] and the oxidation products of **5** and **10** [jatrorrhizine (**15**) and dehydrodiscretine (**16**)]. Assuming that all berbines are formed from (*S*)-norreticuline by a berberine bridge enzyme (73,77,78), this would seem to imply that the formal translocation of a methyl group from the methoxyl at C2 to the C3 hydroxyl group is a practically universal occurrence in this genus. In the rather well-studied genus *Guatteria*, coreximine (2,11-dihydroxy-3,10-dimethoxyberbine, one of the putative precursors of the

whole series) is present in two out of four berbine-accumulating species reviewed two decades ago (51). The fact that only 4 out of 18 *Guatteria* species were shown to contain berbines (and protoberberines were not recorded) suggests that the berberine bridge pathway is considerably less active in *Guatteria* than in *Duguetia*. The presence of spiduxine (**13**) and thaicanine (**14**) in *Duguetia* is another indication of the greater ability of this genus to elaborate the berbine skeleton.

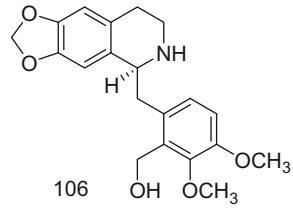
Regarding the (tetrahydro)retroprotoberberine spiduxine (**13**) (21), Shamma proposed in his 1972 treatise on the isoquinoline alkaloids that the related mecambridine, orientalidine, and their oxidation products PO-5 and PO-4 might arise from a berbine by cleavage of the N−C8 bond giving a 1-benzyl-3,4-dihydroisoquinoline that could be reduced to its tetrahydro counterpart, *N*-methylated, and a new ''berberine bridge'' built (80). This scheme is illustrated for the case of spiduxine (**13**) (Scheme 5).

Elegant though this model may appear to be, it lacks experimental support. Considering the ability of *Duguetia* species to introduce one-carbon units in the unexpected C7 position (viz. **53**−**55**), for the sake of parsimony one can also speculate that spiduxine is generated by formylation *ortho* to the phenolic hydroxyl of 2-*O*-methylcoreximine. Nevertheless, a few years ago the unusual structure of a new benzyltetrahydroisoquinoline alkaloid named (+)-argenaxine (**106**) (isolated from *Argemone mexicana*, Papaveraceae) was published (81), with a



Scheme 5 Proposed biogenesis of spiduxine.

regio- and stereochemistry compatible with its hypothetical formation by cleavage of an (*S*)-berbine and the possibility of it being a precursor of a tetrahydroretroprotoberberine (or retroberbine).



106

Interestingly, none of the berbines or protoberberines isolated from *Duguetia* have a methylenedioxy group, suggesting that the enzyme that effects closure of this ring in the many *Duguetia* methylenedioxy-aporphinoids, supposedly a member of the CYP719A subfamily of cytochrome P450s (82), does not accept the geometrically extended berbine skeleton.

The apparently unusual stereochemistry of the morphinandienone (9*S*)-sebiferine (**18**) seems to be justified by the fact that, at least in *Cocculus laurifolius* (Menispermaceae), the biosynthetic conversion of (*S*)- and (*R*)-reticuline (**1**) into sebiferine (**18**) is not stereospecific (83). The C−C phenolic coupling reaction of (*R*)-reticuline (**1**) to salutaridine is the first morphinandienone-forming step, at least in morphine biosynthesis (84), but the enzyme that catalyzes this reaction has not yet been characterized.

Aporphines are believed to be formed by C−C phenolic coupling between C8 and C2′−C6′ of a benzylisoquinoline or, via an intermediate proaporphine, between C8 and C1′. An enzyme catalyzing the first route, CYP80G2, has now been cloned and characterized from *Coptis japonica* (85). This enzyme converts (*S*)-reticuline (**1**) to its direct coupling product (*S*)-corytuberine. If an analogous enzyme is operating in *Duguetia*, it should be responsible for the formation of isocorydine (**41**), an *O*-methylation product of corytuberine and the probably derived norisocorydine (**40**) of *D. vallicola* and *D. furfuracea*. The presence of the proaporphine glaziovine (**19**) in *D. vallicola* is somewhat surprising considering that its likely biogenetic derivatives, (*R*)-aporphines with the 1-hydroxy-2-methoxy, or the 1,10-dihydroxy-2-methoxy, or 1-hydroxy-2,10-dimethoxy substitution patterns seem to be completely absent from the genus.

The 9,11-substitution pattern in the D ring of aporphines is of taxonomic significance in the Annonaceae, as already noted by Roblot *et al*. in 1983 (20). Only one aporphine with this structural feature has been reported in the Ranunculaceae and Menispermaceae (86,87) and these

alkaloids are mainly present in *Guatteria* and *Duguetia* (17,20,21,27, 29,30,50,88). In the review on *Guatteria* alkaloids published in this series (51), it was proposed that one of the ring D substituents might be introduced *meta* to the other, once the aporphine skeleton had been generated from the appropriate proaporphine, stating that either the C11-oxygenated puterine (**31** in this review) or guadiscine (7,7-dimethyl-9-methoxy-1,2-methylenedioxy-6,6*a*-didehydronoraporphine) could be precursors of the 9,11-dioxygenated alkaloids, and that the process might not be very regiospecific. Actually, guadiscine (present in *G. discolor* and *G. melosma*) is only a reasonable precursor of guadiscoline (7,7-dimethyl-9,11-dimethoxy-1,2-methylenedioxy-6,6*a*-didehydronor-aporphine, only found in *G. discolor*), while **31** would be a possible precursor of isocalycinine, discoguattine, oxoisocalycinine, guacolidine, and guacoline, all of which are *Guatteria* alkaloids, and are not isolated from the genus *Duguetia*.

It is intriguing to note that the only American *Duguetia* species known to accumulate a 7-methoxyaporphinoid [pachypodanthine (**78**), in the abundant Amazonian *D. flagellaris*] should grow down to the coast of the Brazilian states of Pará and Maranhão. This part of the South American Gondwana fragment lies opposite to the western reaches of the Gulf of Guinea and Sierra Leone, to which it was formerly attached, and where *D. staudtii* now grows.

In the recent analysis of the anatomical and morphological data of *Duguetia* and closely related genera (6), *D. confinis* and *D. staudtii*, earlier described as *Pachypodanthium*, are placed close to the African species *Duguetia barteri* (Benth.) Chatrou (also formerly *Pachypodanthium*) and *Duguetia dilabens* Chatrou et Repetur (a new species) and to *D. riberensis* of Venezuela, and presumably Colombia. It would be most interesting if the latter plant could be collected and analyzed to determine if it contains 7-methoxylated aporphinoids, like the reasonably well-studied *D. confinis* and *D. staudtii*.

Pachystaudine (**82**) and norpachystaudine (**81**) are said, on the basis of their CD spectra, to have the 6a*S* configuration. This stereochemistry is exceptional for aporphinoids devoid of substituents on ring D, which are generally believed to arise through the dienol−benzene rearrangement of proaporphines derived from (*R*)-coclaurine or norcoclaurine. This apparent anomaly parallels the identification of the (*R*)-9,10-dioxygenated (−)-dicentrine (**39**), from the leaves of an unidentified Amazonian species (36).

It was argued convincingly on the basis of their common 6a*R* configuration (20), that the *N*-formylnoraporphines, found for the first time in *Duguetia* species, cannot be metabolites of *N*-formyl-1-benzyltetrahydroisoquinolines originating from the cleavage of ring C of (14*S*)-berbines as suggested earlier (89). In addition, it was indicated that the

simultaneous presence of *N*-formyl-, *N*-methyl-, and noraporphines, and the accumulation of the latter as major alkaloidal constituents in *D. calycina* and *D. obovata*, pointed to the noraporphines as final biogenetic products (20). Although the precise sequence was not suggested, analogy with the catabolism of *N*-methyl groups in animals allows the sequence aporphine – *N*-formylnoraporphine – noraporphine to be proposed. Noraporphines are therefore likely precursors of the 7- and 4-hydroxynoraporphines, 7-oxo-, and 4,5-dioxoaporphines, and finally the 1-azaaporphinoids (copyrine alkaloids), aristolactams, azaanthraquinones, and their putative derivatives.

The isolated occurrence of duguevalline (**92**) in *D. vallicola* (27) and oxoisocalycinine in *G. discolor* (50) as the only oxoaporphines with the 9,11-dioxygenation pattern is insufficient to suggest any chemosystematic trend. On the other hand, it might be significant that Colombian *D. spixiana* accumulates seven *N*-oxides (five of them aporphine *N*-oxides), while only one each are found in *D. furfuracea*, *D. flagellaris*, and Bolivian *D. spixiana*, and only two in a single *Guatteria* species (*G. sagotiana*) (51).

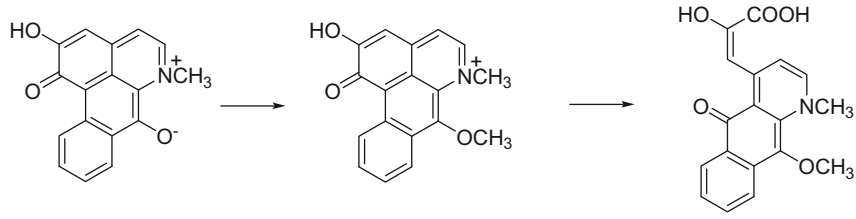
Aminoethylphenanthrenes or secoaporphines are thought to arise by the Hofmann elimination of quaternary aporphine alkaloids (the quaternization and elimination products are commonly termed ''methines''), and this indeed would seem to be the case for atherosperminine (**94**, nuciferine methine) and methoxyatherosperminine (**96**, 3-methoxynuciferine methine). The formation of atherosperminine *N*-oxide (**95**) appears to follow an important catabolic trend for Colombian *D. spixiana*. Noratherosperminine (**93**) would presumably arise through the *N*-demethylation of atherosperminine (**94**), probably catalyzed by a cytochrome P450. An alternative explanation would involve an anomalous Hofmann elimination reaction of the tertiary nuciferine (necessarily in its *N*-protonated form?). Although such a reaction has been documented *in vitro* for boldine (90) in refluxing ammonium acetate solution, it seems extremely unlikely that it should occur nonenzymatically *in vivo*. Therefore, one would have to assume the existence of a ''Hofmannase'' for which there does not seem to be any precedent.

It is interesting that only nuciferine and 3-methoxynuciferine are involved in the biogenesis of these aminoethylphenanthrenes. Nornuciferine (**22**) and 3-hydroxynornuciferine (**25**) have been shown to accumulate only in Bolivian *D. spixiana*, and the former also in *D. flagellaris*, but their tertiary and quaternary analogs, the expected precursors of their ring-opened products, have not been recorded for any *Duguetia* species. This seems remarkable in view of the presence of the close nuciferine congener anonaine (**23**) in Bolivian *D. spixiana* (and also *D. furfuracea*), but not its *N*-methyl homolog roemerine, its quaternary

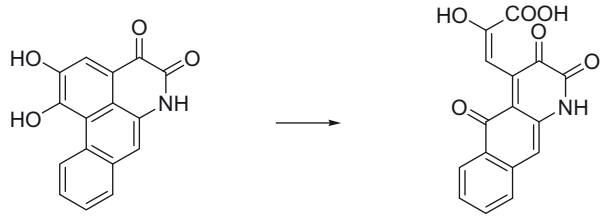
derivative, or its *seco* counterpart. In all, 26 aporphines *sensu stricto*, including several nor- and two quaternary aporphines, are listed above, and only two of them can be envisioned as precursors of the *Duguetia* aminoethylphenanthrenes. On the other hand, the quaternary *N*-methylglaucine (**36**, from *D. furfuracea*) and *N*-methylguatterine (**66**, from *D. odorata*) do not seem to undergo ring opening in this genus. The phytochemical literature records a large number of aminoethylphenanthrenes, many from different Annonaceous genera, apparently derived from aporphines with most of the various substitution patterns. Therefore, the very limited occurrence of these alkaloids in *Duguetia* suggests the hypothesis that they are the products of a metabolic route involving a highly specific enzyme at some key step, possibly the ''Hofmannase'' mentioned above.

The copyrine alkaloids or 1-azaaporphinoids can be viewed as aporphine derivatives in which ring A has been opened (e.g., by extradiol cleavage of a 1,2-catecholic aporphine between C1 and C11b) with subsequent reclosure through condensation with an ammonia molecule (91). Taylor's biogenetic proposal deriving the azafluoranthene, diazafluoranthene, tropoloisoquinoline, 1-azaanthracene, and azafluorenone alkaloids from 1,2-dihydroxy-7-oxoaporphine (liriodendronine) through an initial ring A cleavage (92,93) has been extended to explain the formation of the hadranthines and imbilines via formal 1,4-hydrogenation of the ketoimine function and stabilization by *O*-methylation, either preceded, or followed by, conversion of pyridine ring B to the β-ketolactam function (16). An alternative pathway to the 7-methoxylated 1-aza-4,5-dioxoaporphinoids or the 4,5-dioxocopyrines of *D. hadrantha*, not requiring a reduction step, might start from *N*-methylliriodendronine, in which the C7 oxygen function is already a phenoxy group, particularly in view of the presence of many 7-hydroxy- and two 4-hydroxy-7-methoxyaporphines in *Duguetia* (Scheme 6).

The proposal for the late oxygenation of C4 and C5 could be circumvented by a parallel route to the 4,5-dioxocopyrines starting from 1,2-dihydroxy-4,5-dioxoaporphine, which leaves open the possibility of a monooxygenase-catalysed hydroxylation at C7 (Scheme 7).



Scheme 6 Initial steps of a proposed biogenetic pathway to 4,5-dioxocopyrines starting from *N*-methylliriodendronine.



Scheme 7 First step of a proposed biogenetic pathway to 4,5-dioxocopyrines starting from 1,2-dihydroxy-4,5-dioxoaporphine.

A biogenetic proposal to account for the formation of azahomoaporphines was published 20 years ago in this series (51). According to that hypothesis, spiguetine (**102**) and spiguetidine (**103**) of the Bolivian sample of *D. spixiana* might be derived from the 7-hydroxyaporphines oliveridine (**68**) and roemerolidine (**69**), which are the major alkaloids of the same plant.

It was suggested that α-aroylpyridine derivatives, and more specifically 1-azaanthracene-9,10-diones, such as cleistopholine (**104**), might undergo decarbonylation catalyzed by a metalloenzyme (93). This has now received indirect support from the formation of metal complexes of liriodenine (**83**) which confirm the metallophilicity of the 7-oxoaporphine arrangement of a pyridine nitrogen and a carbonyl oxygen and, presumably, of related systems (94).

Some striking resemblances in the alkaloid chemistry of *Duguetia* and *Guatteria* were pointed out by Cavé in 1984, as indicating the possible proximity of these genera (95). At that time, it was known that both *Duguetia* and *Guatteria* species accumulate 7-alkylated aporphinoids and *N*-formylnoraporphines. It was then suggested that the unusual oxazine-condensed aporphine system of duguenaine (**53**) and duguecalyne (**54**) might arise from ring closure of *N*-formyl-7-methylaporphinoids or, alternatively, their 7-formyl-*N*-methyl counterparts, indicating that such potential intermediates had already been found in *D. spixiana* (duguespixine, **55**) and *Guatteria trichostemon* (trichoguattine, the 1,2-methylenedioxy analog of **55**). In fact, the related 9-hydroxylated belemine and goudotianine have also been isolated from a couple of *Guatteria* species (96,97). Another common feature pointed out by Cavé was the 9,11-dioxygenation pattern of some *Duguetia* and *Guatteria* aporphinoids. At that time (1984), he noted that the phenol function is located at C9 in *Guatteria* and at C11 in *Duguetia*. This is not strictly so, as discoguattine, guacoline and guadiscoline are 9,11-dimethoxylated aporphinoids, but the first two alkaloids could obviously be formed by *O*-methylation of their putative 9-hydroxy precursors isocalycinine and guacolidine.

It is worth mentioning that *D. calycina* and *D. spixiana*, the only *Duguetia* species known to contain 1-aminoethylphenanthrenes, are classed in the section *Sphaerantha*, and thus the occurrence of this small group of alkaloids might be of chemosystematic significance. Interestingly, atherosperminine, *N*-oxyatherosperminine, noratherosperminine, together with the 2-*O*-demethylated atherosperminine analog argentinine (*N*-methylasimilobine methine), are the only 6,6*a*-secoaporphines isolated from the larger Annonaceous genus *Guatteria*, and that from the single species *G. discolor* (50,98). However, *G. discolor* appears to have arisen from fairly recent (Pliocene or Pleistocene) diversification events within *Guatteria* (99), placing it at a considerable evolutionary distance from the Eocene split that presumably originated *Duguetia* (46), and suggesting that aminoethylphenanthrene accumulation is not an ancestral character, but rather one that has appeared in a scattered fashion in plants that synthesize aporphines, either by convergent evolution or by cross-colonization by endophytic fungi with the relevant synthetic abilities. As in *Duguetia*, the *Guatteria* aminoethylphenanthrenes are formally and exclusively derived from ring D-unsubstituted aporphines. As in the case of the copyrine alkaloids, it has been proposed that the azahomoaporphine skeleton arises by oxidative cleavage of the aporphine system, in this case between C6a and C7, and reclosure incorporating an ammonia molecule (100). Finally, if staudine (**105**) is in fact an enzymatic product, one would have to invoke catalysis by a Diels–Alderase to explain its formation.

A striking aspect of the known alkaloid chemistry of *Duguetia* is the apparent lack of correlation between the structures of the isolated alkaloids and the morphologically based classification of the genus into sections. Although the large section *Duguetia*, for example, seems to be well-supported on morphological and genomic grounds, none of the (relatively few) individual alkaloids isolated from *D. odorata* and *D. stelechantha* have been found in the seemingly exhaustive studies of *D. furfuracea*, classed in the same section. One would like to find a more convincing degree of chemosystematic order in such an extensively studied genus, but this will probably be impossible without more exhaustive studies of some species, and adequate quantification of the individual alkaloids in crude extracts rather than the isolated yields, probably using a metabolomic (or metabonomic, or metabolic profiling) approach (101,102). With a significantly more complete picture, it should become possible to reasonably address the fascinating question of how the diverging biosynthetic pathways present in *Duguetia* are regulated.

VI. ETHNOPHARMACOLOGY AND PHARMACOLOGY

Surprisingly little has been published on the ethnopharmacology of *Duguetia* species, as recognized by the authors of one of the most recent papers discussed here (13). A possible explanation is that most of these plants grow in the Amazon region and, if they have medicinal or related uses, are only employed by ethnic groups whose practices have been poorly recorded by outsiders. As is the case with the bulk of ethnopharmacological data, traditional uses are frequently difficult or impossible to ascribe to medical conditions recognized by Western science, and even less so to pharmacological mechanisms. Moreover, in the vast majority of instances, the effectiveness of these practices has not been substantiated scientifically through direct observation. Furthermore, the literature reveals an unfortunate tendency to ascribe a biological activity of a plant or a plant extract, obtained with little regard to the traditional mode of preparation, to whatever can be isolated (and often, but not always, biologically evaluated). Finally, there is an almost complete absence of the quantitative analysis of the active constituents, which can lead to the erroneous conclusion that a substance present in insufficient amounts to produce any effect is responsible in the field for test results obtained with the pure compound.

D. furfuracea has two recorded uses in traditional medicine: its powdered seeds are mixed with water and used to kill lice, and an infusion of the twigs and leaves is used against rheumatism (13). *D. flagellaris* is also used to treat rheumatism as an infusion in sugar cane spirit (30,103). *Duguetia glabriuscula* is said to be used to kill cockroaches, although the report does not mention what part of the plant is insecticidal (104). The insecticidal uses of *Duguetia* species are probably not related to their alkaloid content, but rather to the presence of the so-called ''Annonaceous acetogenins,'' characteristic of many Annonaceae, but not yet reported for the genus *Duguetia*. It is worth noting that the use of powdered Annonaceae seeds as insecticides was first recorded four centuries ago (105).

D. confinis is used in tropical Africa as a cough suppressant and analgesic, particularly for toothache (37). The stem bark of *D. staudtii* is used by some populations in the Ivory Coast as an arrow poison ingredient. The bark is also frequently used in traditional medicine for several indications: ground to a pulp with kola nut it is used to treat gastrointestinal pain and locally, mixed with *Ficus exasperata* leaves, as an anti-inflammatory; it is also considered an analgesic, and some populations in the Congo use it for cough, and for difficulty in breathing. The Pomo tribe, also in the Congo, claims that the bark of this species is a purgative and an aphrodisiac (39).

No ethnopharmacological data seem to have been published for any other *Duguetia* species. In contrast, although information is lacking regarding the pharmacology of most individual *Duguetia* alkaloids, the last two decades have seen an extraordinary number of papers on the biological properties of a few alkaloids that are either abundant, characteristic, or recognized as active principles of other plants, and are also present in *Duguetia*. Additionally, some generalizations can be made safely as to the related pharmacological activities of substances that are close structural congeners.

A. Benzyltetrahydroisoquinolines

(*S*)(+)-Reticuline (**1**) is a dopamine receptor antagonist, blocking the actions of the dopamine agonist apomorphine, causing decreased locomotor activity and producing catalepsy in rats (106,107). These effects seem to be elicited by the blockade of postsynaptic striatal dopamine receptors (108). Dopaminergic antagonism by reticuline (**1**) appears to be rather weak, however, and has not attracted much interest, although it might be involved in the central depressant effects observed in rats and mice (109). Reticuline (**1**) inhibits dopamine uptake and at high concentrations is toxic to dopaminergic and GABAergic neurons. It has therefore been suggested that it might be involved in the genesis of the atypical Parkinsonism of the French West Indies, associated with the consumption of fruit and infusions of the reticuline-containing *Annona muricata* (110). (*S*)(+)-Reticuline (**1**) is also a weak neuromuscular (nicotinic cholinergic) blocker (111). In addition, it reduces the contractile force of guinea pig heart by blocking calcium channels (112). (*S*)(+)-Reticuline-induced uterine relaxation and vasorelaxation by L-type Ca^{2+} channel blockade have also been demonstrated (113,114). Nevertheless, the cardiovascular effects of reticuline (**1**) appear to depend on the blockade of Ca^{2+} entry and on the inhibition of Ca^{2+} release from norepinephrine-sensitive intracellular stores, and by cholinergic (muscarinic) stimulation and nitric oxide synthase activation in the vascular endothelium (115).

(*S*)(+)-Reticuline (**1**) has antiplatelet aggregation activity (116). It shows some antifungal activity (117), and is rather weakly antiplasmodial (118). It is also claimed to accelerate hair growth (119).

Reticuline, at 20 mg/kg, administered intraperitoneally, is significantly antinociceptive in the acetic acid-induced mouse writhing test, and quenches diphenylpicrylhydrazyl (DPPH) radicals with a scavenging concentration (SC_{50}) of 47 μg/mL (143 μM) (120). The latter antioxidant property could well be related to its effects on inflammation and pain.

Nothing is known about the pharmacology of *N*-oxycodamine (**2**) or, in fact, of other benzyltetrahydroisoquinoline *N*-oxides.

B. Bisbenzylisoquinoline

Isochondodendrine (**3**) was mentioned more than 50 years ago as a possible agent for the treatment for dysmenorrhea, but this lead does not seem to have been pursued (121,122). The only recent work found refers to the potent antiplasmodial activity of isochondodendrine (**3**) *in vitro* (IC_{50}=0.10 μg/mL) (123,124), which makes one wonder if *D. furfuracea* might be used to treat fever or, more specifically, malaria, in the area where it grows.

C. Berbinoids

Discretamine (**4**) is a potent α_1-adrenergic blocker, comparable in potency and basic pharmacology to the hypotensive drug phentolamine. It also blocks α_2-adrenoceptors and 5-HT_2 serotonin receptors, at several times higher concentrations, and seems to be devoid of action at acetylcholine, histamine, leukotriene, thromboxane, prostaglandin $F_{2\alpha}$, or angiotensin II receptors (125). Its action on α_1-adrenoceptor subtypes is selective for α_{1D} over α_{1A} and α_{1B} (126). Discretamine (**4**) antagonizes the contraction of human hyperplastic prostate tissue elicited by phenylephrine, electrical stimulation, or high Ca^{2+} (127). Its antiplatelet aggregation effect is another potential beneficial action of this alkaloid (128). Discretamine (**4**) is hypotensive in rats at doses between 0.01 and 10 mg/kg. A series of *in vitro* experiments suggests that the hypotensive effect of discretamine (**4**) is probably due to peripheral vasodilation related to nitric oxide release from the vascular endothelium (34).

Of all the berbine alkaloids recorded as *Duguetia* constituents, THP (**7**) is by far the most studied in relation to its pharmacology, probably because its (*S*)(−)-enantiomer (rotundine) and the racemic mixture are active constituents of the Asian drugs *Stephania rotunda* and *Corydalis racemosa*, respectively. As far back as 1970 (*S*)-THP, with the generic name ''gindarin,'' was evaluated for dermatological use in the treatment of neurodermatitis and alopecia areata, but this study does not seem to have progressed any further (129).

(±)-THP (**7**) is listed in the Chinese Pharmacopoeia as an analgesic with sedative–hypnotic effects. This alkaloid, together with its close analogs tetrahydroberberine and tetrahydrocoptisine, though apparently not tetrahydrojatrorrhizine (**5**), were shown to exhibit central depressant effects in mice and rats similar to those of the well-known neuroleptic chlorpromazine, leading to the suggestion that these berbines might represent ''a new type of tranquilizer'' (130). (±)-THP (**7**) was later

recognized as a dopamine, and, to a lesser extent, noradrenaline and serotonin depletor with an action similar to reserpine (131). In the former Soviet Union, the *S*-enantiomer, ''gindarin,'' was subjected to a preclinical study (in rats) in the framework of its possible use as a tranquilizer (or neuroleptic), and was found to be embryotoxic (132). (*S*)-THP (**7**) was subsequently shown to be a dopamine antagonist, while the *R*-isomer appears to be responsible for dopamine depletion (133,134), acting on both pre- and postsynaptic receptors (135). These dopaminergic actions probably explain the neuroleptic-like activity of both (*S*)- and (±)-THP (**7**). Radioligand displacement studies showed that (*S*)(−)-THP (**7**), but not its enantiomer, has affinity for D_2(-like) receptors (136). Subsequently, *in vivo* data were acquired showing that this alkaloid lacks agonistic effects (137). It has been shown recently that (*S*)(−)-THP (**7**) binds with high affinity (K_i=94 nM) to rat D_1 dopamine receptors, while a 3:1 mixture, in which the *R*-enantiomer predominates, has only micromolar affinity (138).

(±)-THP (**7**) decreases motor activity in rats, producing rigidity (or catalepsy?) at higher doses, apparently due to enhanced turnover of dopamine, although increased turnover is also observed for norepinephrine and, at higher doses, for serotonin (139). The antinociceptive action of (*S*)(−)-THP (**7**) is attributed to its D_2 antagonism in the striatum and nucleus accumbens, thus enhancing the activity of the brainstem descending pain modulation system (140–142). This effect might be reinforced by endogenous opioid release, as chronic administration of the alkaloid increased the Leu-enkephalin content in the rat striatum (143), and lesion of a predominantly β-endorphin pathway abolished the analgesic action of (*S*)-THP (**7**) (144). The hypotensive and heart rate-slowing effects of (±)-THP (**7**) have also been related to D_2 antagonism (145). Nevertheless, other mechanisms are clearly at work in the cardiovascular actions of this alkaloid, whether the *S* isomer or the racemic mixture. Calcium channel blockade and α_1 and α_2 adrenoceptor antagonism were first implicated in 1989 (146). (*S*)-THP (**7**) is also a subtype nonselective α-adrenoceptor antagonist (147). Experiments in rats demonstrated the protective effects of the *S*-enantiomer in experimental myocardial infarction, apparently related to its action on calcium channels (148,149). The first clinical results showing the effectiveness of (*S*)(−)-THP in patients with atrial fibrillation or paroxysmal tachyarrhythmias were published in 1993 (150,151). (±)-THP (**7**) is used for the treatment of pain, but reports have surfaced of severe cardiac and neurological toxic effects from abuse of this drug, and it has been suggested that these problems are also due to calcium channel blockade (152). Although the peripheral effects on calcium channels and adrenergic receptors are supported by later studies, there are strong indications that the cardiovascular effects of (±)-THP (**7**) are due, at least

in part, to hypothalamic dopamine antagonism and/or 5-HT_2 serotonergic agonism (153,154). The racemic mixture also induces hypothermia, which is attenuated by brain serotonin depletion or 5-HT_2 serotonergic receptor activation, again indicating a central serotonin antagonist action of the drug (155).

Pretreatment with (±)-THP (**7**) suppresses behavioral activation by picrotoxin (a noncompetitive $GABA_A$ receptor inhibitor) in rats, suggesting that this alkaloid might suppress epileptic seizures through inhibition of dopamine release (156). In this connection, the alkaloid was tested on the development of seizures in animals with electrically kindled amygdala, and found to be very effective as an antiepileptogenic and anticonvulsant agent in this model (157). It was subsequently shown that THP (**7**) is a positive allosteric modulator of $GABA_A$ receptors, thus sharing some of the pharmacological properties of the antiepileptic barbiturates and benzodiazepines (158). An independent study showed that orally administered (±)-THP (**7**) exhibits anxiolytic-like actions in mice, and that these effects are abolished by coadministration of a benzodiazepine antagonist, suggesting that THP interacts with the benzodiazepine site of the $GABA_A$ receptor (159).

In rats, (*S*)(−)-THP (**7**) inhibits methamphetamine- and cocaine-induced conditioned place preference, a preliminary test of possible antiaddictive activity in humans (160,161). Furthermore, it reduces cocaine self-administration and reinstatement, suggesting that it could also be useful in the treatment of cocaine addiction (162,163). Studies in rodents and in humans suggest that (*S*)(−)-THP (**7**) can ameliorate opioid drug craving and increase abstinence (164−165).

THP (**7**) is a weak inhibitor of the mitochondrial respiratory chain (166), and binds poorly to DNA (dissociation constants of the order of 10^{-4} M, with the *R*-enantiomer binding about twice as strongly as the *S*-enantiomer) (167). In line with these results, THP (**7**) and also xylopinine (**12**) are only weakly cytotoxic (168).

Other miscellaneous effects of THP (**7**) have been examined in relatively little detail. The racemic alkaloid produces significant decreases in thyroid function in hyperthyroid rats, apparently by inhibiting the release of thyrotropin-stimulating hormone (169). (±)-THP (**7**) attenuates several parameters related to neuronal damage caused by heatstroke in rats (170). (*S*)-THP (**7**) has several beneficial actions during acute cerebral ischemia-reperfusion in rats (171−174), and depresses the expression of adhesion molecules induced by lipopolysaccharides, suggesting that it might be useful in the treatment of inflammation (175). In this connection, and considering that free radicals are involved in inflammation, it should be pointed out that THP (**7**) exhibits antioxidative activity of similar potency to phenolic flavonoids in the lipid peroxidation and hemolysis assays (176). The racemic alkaloid

protects against carbon tetrachloride-induced liver damage in mice, which is also related to the formation of free radicals (177). THP (**7**) causes paralysis in the domestic fowl parasitic worm *Raillietina echinobothrida* at 1, 2, and 5 mg/mL, apparently related to disturbance of the nitric oxide signaling pathway (178).

The antiplasmodial activity of thaicanine (**14**) was demonstrated almost two decades ago, at low-to-submicromolar concentrations, against the chloroquine-sensitive *Plasmodium falciparum* D-2 strain and the resistant W-2 strain (120). Discretine (**10**) inhibits the growth of *P. falciparum* (chloroquine-resistant FcB1/Colombia strain) with IC_{50}= 1.6 μM, and is practically noncytotoxic against KB cells (179). THP (**7**) and xylopinine (**12**) are only weakly active against *P. falciparum* (IC_{50}=32 and 52 μM, respectively) (180).

D. Protoberberines

Jatrorrhizine (**15**), only isolated to date, in the Annonaceae, from *D. trunciflora*, is mentioned in a large number of pharmacological papers. Jatrorrhizine (**15**) lowers arterial blood pressure in normotensive dogs (181). It blocks α_1 and α_2 adrenergic receptors with moderate potency and exhibits some antihypertensive and heart rate-slowing activity in rats, although at higher concentrations these effects are reversed (182). Jatrorrhizine (**15**) inhibits both monoamine oxidase isoforms (MAO-A and MAO-B) of rat brain with IC_{50} values of 4 and 62 μM, respectively (183). It also inhibits rabbit platelet aggregation *in vitro* (184), and acetylcholinesterase inhibition by jatrorrhizine (**15**) has also been reported (185).

Antimicrobial activity of jatrorrhizine (**15**) against *Mycobacterium smegmatis* was demonstrated at concentrations of less than 100 μg/mL (184). It was recently tested against a panel of human dermatophytes and yeast-like fungi, exhibiting minimal inhibitory concentrations (MIC) between 62.5 and 125 μg/mL against *Epidermophyton*, *Trichophyton*, and *Microsporum* species, and 250 and 500 μg/mL against *Candida tropicalis* and *Candida albicans*, respectively; all better results than those obtained with berberine. However, it was inactive against *Scopulariopsis brevicaulis* (186). Bifonazole and fluconazole were used as positive controls, the former exhibiting MIC values above 100 μg/mL for all strains, but *Epidermophyton floccosum*, and the latter also, with the additional exception of *Trichophyton rubrum*. Tests against 20 strains of *Staphylococcus* (including 14 of *S. epidermidis*) and 20 strains of *Propionibacterium acnes*, and 20 *Candida* strains (including 17 of *C. albicans*) showed that the antibacterial potency of jatrorrhizine (**15**) is less than that of berberine, and that both alkaloids are inferior to commonly used antibacterial drugs. However, jatrorrhizine (**15**) may be a good lead for the

development of more effective antifungal agents than those in current use (187).

This alkaloid (**15** is active) *in vitro* against two different clones of *P. falciparum* with IC_{50} values of 0.422 and 1.607 μg/mL, potencies comparable to that of quinine, however, in an *in vivo* (mouse) screen against *Plasmodium berghei* it was inactive (188). Against the *P. falciparum* multidrug-resistant strain K1, it exhibited IC_{50}=3.15 μM, (corresponding to 1.1 μg/mL), and showed very modest activity against *Entamoeba histolytica* (189). In cultures of *Babesia gibsoni*, an important parasite in dogs and a member of a genus causing babesiosis in other carnivores, ruminants, and horses, jatrorrhizine (**15**) inhibited growth at low-to-moderate concentrations (190). Dehydrodiscretine (**16**) inhibits the growth of *P. falciparum* with IC_{50}=0.64 μM (multidrug-resistant K1 strain) (189), and 0.9 μM (chloroquine-resistant FcB1/Colombia strain) (179).

Jatrorrhizine (**15**) and dehydrodiscretine (**16**) have negligible cytotoxicity against KB cells (179,189). The interaction of jatrorrhizine (**15**) with DNA resembles that of ethidium bromide, the classical DNA intercalator (191). Binding to calf thymus DNA reveals two different binding sites with dissociation constants of about 25 and 35 μM (192). Binding to the double-stranded oligodeoxynucleotide *d* $(AAGAATTCTT)_2$ shows both 1:1 and 1:2 stoichiometries, with similar affinity to that of palmatine, and greater than those of coptisine or berberine (absolute values were not determined) (193). Similar studies with different sequences indicated that the affinity of jatrorrhizine (**15**) was reduced for *d*$(AAGGATCCTT)_2$ and *d*$(AAGCATGCTT)_2$ relative to the other protoberberine alkaloids tested (194). Finally, using competitive ethidium bromide displacement experiments on calf thymus DNA and synthetic double-stranded polynucleotides, the higher affinity of jatrorrhizine (**15**) relative to palmatine and berberine and their preference for AT-rich DNA were confirmed (195). In an eukaryotic test model (*Euglena gracilis* vs. the direct-acting mutagen acridine orange), jatrorrhizine (**15**) exhibited weak antimutagenic activity (196).

Jatrorrhizine (**15**) was shown to be a weak scavenger of DPPH radicals, and a modest inhibitor of lipid peroxidation in unilamellar dioleyl-phosphatidylcholine liposomes (197). It downregulates tumor necrosis factor alpha (TNFα) and E-selectin expression, and decreases the content of thromboxane B(2) in rat intestinal microvascular endothelial cells, suggesting that it might reduce inflammatory response by affecting cytokines and autacoids (198,199), rather than by virtue of its poor antioxidant properties.

Single doses of 50 and 100 mg/kg jatrorrhizine (**15**) decreased blood glucose in normal and alloxan-diabetic mice and increased succinate dehydrogenase activity in the liver, however, it had no effect on blood

lactic acid or liver lactate dehydrogenase. The alkaloid also decreased liver glycogen in normal mice, suggesting that its hypoglycemic activity can be attributed to increased aerobic glycolysis (184). Several methods have been used to study the binding of jatrorrhizine (**15**) to human serum albumin, concluding that the protein's secondary structure is altered and hydrophobic and electrostatic interactions play a major role (200).

Pseudopalmatine (**17**) does not seem to have been studied pharmacologically.

E. Glaziovine (19)

In the early 1970s the pharmacology of glaziovine (**19**) was explored by an Italian pharmaceutical company that registered it as a tranquilizer under the trademark Suavedol®. Its psychopharmacology was compared with that of diazepam in a double-blind clinical trial (201), and its human pharmacokinetic parameters were studied (202). In addition, it was reported to possess anti-gastric ulcer properties in rodents and in humans (203,204). No studies appear to have addressed its mechanisms of action as either an anxiolytic or antiulcerogenic agent.

More recently, glaziovine (**19**) was evaluated for anti-hepatitis B virus activity. This alkaloid proved to be highly potent, as judged by its IC_{50} value of 8 μM, as an inhibitor of HBV surface antigen production. The corresponding value for the positive control, the anti-HBV drug 3TC or Lamivudine, was 11.7 mM. However, glaziovine (**19**) was more toxic to uninfected that to infected cells (205).

The isolated yield of glaziovine (**19**) from *D. vallicola* leaves was 0.27%, placing this abundant and easily accessible material in a good position as a source of a useful plant drug (26). Glaziovine (**19**) is one of 60 alkaloids listed as having particular pharmaceutical and biological significance (206).

F. Aporphines

Anonaine (**3**) relaxes rat aorta and tail artery precontracted with noradrenaline, predominantly through adrenergic receptors. Since its affinity for L-type Ca^{2+} channels is an order of magnitude less for α_1 adrenoceptors in rat cerebral cortical membranes, it does not contribute to intracellular mobilization of Ca^{2+}, and its effect on phosphodiesterases is negligible. It is also slightly selective for α_{1A} and α_{1D} adrenoreceptors relative to the α_{1B} subtype, as determined by radioligand competition experiments (207,208).

Xylopine (**28**) is a selective α_1 (vs. α_2) adrenergic receptor antagonist with submicromolar functional potency (209). In the rabbit oviduct, isocorydine (**41**) inhibits spontaneous and noradrenaline-elicited

contractions, indicating that this alkaloid is an adrenoceptor antagonist (210). A further study in a rat aorta model suggested that the effect is mediated primarily through α_1 adrenoceptors (211). The effects of isocorydine (**41**) on the action potentials of canine heart muscle cells have also been studied *in vitro* (212).

Asimilobine (**20**) inhibits rabbit aortal contractions induced by 10^{-6} M serotonin with pA_2=5.78, suggesting that this alkaloid is a 5-HT_2 serotonin receptor antagonist (213). Dicentrine (**39**) inhibits the contraction of rat stomach muscle strips induced by serotonin, histamine, K^+, and Ca^{2+} in a noncompetitive manner. In the case of serotonin-induced contractions, the relaxation depends on Ca^{2+} release from intracellular stores, suggesting that 5-HT (presumably 5-HT_{2B}) receptors are involved (214). Asimilobine (**20**), nornuciferine (**22**), and anonaine (**23**) bind to 5-HT_{1A} serotonin receptors with low micromolar IC_{50} values versus [^{3}H] rauwolscine, and were shown to be full agonists (215). In [^{3}H]8-hydroxy-2-(di-*N*-propylamino)tetralin displacement experiments, *N*-methyllaurotetanine (**37**) exhibits high affinity for 5-HT_{1A} receptors (K_i=85 nM, pK_i=7.07) (216).

Isoboldine (**38**) relaxes isolated guinea pig trachea with IC_{50}=710 μM, suggesting a β-adrenoceptor-mediated mechanism (217). Dicentrine (**39**) has been extensively studied as a cardiovascular agent. It was first shown to be a potent α_1-adrenoceptor antagonist (less potent than prazosin, and more potent than phentolamine) with little effect on β-adrenergic receptors (218,219). Its hypotensive effect was demonstrated *in vivo* in rats by the intravenous and oral routes, and in conscious, spontaneously hypertensive animals, oral administration of 5 and 8 mg/kg caused hypotension lasting more than 15 h (220). In rats fed a high-cholesterol diet, oral administration of dicentrine (**39**) decreased the mean arterial pressure (more so in spontaneously hypertensive animals), and reduced the total plasma cholesterol by reducing the low-density lipoprotein fraction, and the total plasma triglyceride by reducing the very low-density lipoprotein fraction (221).

Experiments in isolated cardiac cells and in rabbit heart showed that dicentrine (**39**) blocks sodium and potassium currents, and is a potentially useful antiarrhythmic agent at doses in the same range as quinidine (222,223). The effects of dicentrine (**39**) on the mechanical properties of systemic arterial trees have been studied in dogs (224). Dicentrine (**39**) inhibits serum-stimulated kidney mesangial cell proliferation in the rat, and was therefore viewed, together with other vasodilators, as an agent with the potential to delay the progression of chronic glomerulopathy (225). As an α_1-adrenoceptor antagonist it also inhibits contractions of human hyperplastic prostate elicited by adrenergic stimulation, and might therefore be of use to relieve bladder outlet obstruction in patients with benign prostatic hyperplasia (226).

Anonaine (**23**) and isopiline (**24**) inhibit dopamine uptake by rat striatal synaptosomes with IC_{50}=0.8 and 2.5 μM, respectively. Anonaine (**23**) is a selective uptake inhibitor relative to its affinities for D_1-like and D_2-like dopamine receptors as determined in radioligand displacement experiments (IC_{50} vs. [^{3}H]SCH23390 and [^{3}H]raclopride: 68 and 19 μM, respectively; ratios of uptake to receptor binding IC_{50} values: 85.0 and 23.5), while isopiline (**24**) exhibits much lower selectivity (D_1-like and D_2-like binding IC_{50}: 10 and 34 μM, respectively; IC_{50} ratios 3.0 and 13.6) (227).

Asimilobine (**20**), in the 0.05−0.2 μM range, reduces intracellular dopamine in PC12 cells for 24 h with IC_{50}=0.13 μM. At this concentration it decreases the activities of tyrosine hydroxylase (TH, by 73.2% and for a longer time) and aromatic L-amino acid decarboxylase, and reduces TH mRNA and intracellular cAMP levels. Alone, it does not alter PC12 cell viability at concentrations up to 5 μM. However, in association with L-DOPA asimilobine (**20**) inhibits the L-DOPA-induced increase in dopamine levels and enhances L-DOPA cytotoxicity (228).

N-Methylasimilobine (**21**) is a significant inhibitor of platelet aggregation elicited by collagen, arachidonic acid (AA), and platelet-activating factor (PAF). Xylopine (**28**) and *N*-methyllaurotetanine (**37**) inhibit platelet aggregation with different potencies depending on the substance used as an aggregation inducer in each case (229). Dicentrine (**39**) also inhibits platelet aggregation induced by AA, collagen, adenosine diphosphate (ADP), PAF, thrombin, or the synthetic U46619, and induces ATP release from platelets. Additional experiments indicated that these effects are due to the inhibition of thromboxane B2 formation and increased cAMP levels (218,230,231).

N-Methyllaurotetanine (**37**), administered intravenously, is antihyperglycemic in normal and streptozotocin-induced diabetic rats (232). *N*-Methyllaurotetanine (**37**) and norisocorydine (**40**), at 20 mg/kg i.p., are significantly antinociceptive in the acetic acid-induced mouse writhing test, and quench DPPH radicals with SC_{50}=28 and 14 μg/mL (82 and 43 μM), respectively (25,120). Antinociceptive activity is often associated with free radical inactivation, and in this regard it should be mentioned that anonaine (**23**) was one of the first aporphine alkaloids for which antioxidative activity was demonstrated (233).

Anonaine (**23**) reduces the viability of normal rat hepatocytes, and HepG2 and HeLa tumor cells, with IC_{50} values of 70.3, 33.5, and 24.8 μg/mL, respectively, in 24-h experiments (234). Non-cancer Vero and MDCK cells exposed to 100 μM anonaine (**23**) for 24 h experienced reduced viability by about 25% and 5%, respectively (235). In the case of HeLa cells, the decrease amounted to 77%, and was associated with DNA damage and a dose-related block of the cell cycle before the G_1 phase. These effects were correlated to increased intracellular nitric oxide,

reactive oxygen species, glutathione depletion, disruption of the mitochondrial transmembrane potential, activation of caspases 3, 7, 8, and 9, and poly(ADP-ribose) polymerase (PARP) cleavage with up-regulation of Bax and p53 proteins (235).

Dicentrine (**39**) inhibits the growth of murine leukemia P388 and L1210, melanoma B16, bladder cancer MBC2, and colon cancer Colon 26 cells in culture, and also reduces mitogen-induced lymphocyte proliferation and the growth of IL-dependent CTLL2 cells (236). It slows the growth of the human hepatoma cell line HuH-7 and decreases the efficiency of colony formation by these cells and the MS-G2 line, and strongly inhibits DNA and RNA synthesis. Additional evaluations in 21 tumor cell lines showed that dicentrine (**39**) was particularly cytotoxic to esophageal carcinoma HCE-6, lymphoma Molt-4 and CESS, leukemia HL60 and K562, and hepatoma MS-G2 (237). This alkaloid is active in a DNA unwinding assay, and is a modest inhibitor of topoisomerase II (IC_{50}=27 μM) (238). However, it shows no antiproliferative activity versus several yeast strains (239). Duguetine (**76**) ''caused considerable antitumoral activity'' (240).

An extract of *D. odorata* was found to inhibit the G_2 DNA damage checkpoint, a target that is expected to enhance the effectiveness of DNA-damaging anticancer therapy. Dehydrodiscretine (**16**), pseudopalmatine (**17**), oliveroline (**60**), and *N*-methylguatterine (**66**), were isolated by bioassay-guided fractionation following this bioactivity, however, only oliveroline (**60**) had confirmed, though modest, potency (at concentrations above 10 μM), and was isolated in sufficient amounts for additional testing (14).

Pachystaudine (**82**) interferes with the replicative cycle of herpes simplex virus type 1 (HSV-1) (241).

Anonaine (**23**) and xylopine (**28**) are weakly antibacterial and antifungal (120,242,243), and anolobine (**27**) is only active against Gram-positive bacteria and *Mycobacterium phlei* in the 10^{-4} molar range with MIC_{90}=12–50 and 6–25 μg/mL, respectively (243). Anolobine (**27**) induces chromosomal aberrations in a Chinese hamster lung cell line at concentrations as low as 2.5 μg/mL (244). At 300 μg/mL, dicentrine (**39**) showed ''moderate'' to ''good'' activity against the fungi *Microsporum canis*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, and *E. floccosum*, but was inactive against *C. albicans*, *Aspergillus niger*, and *Penicillium* sp. (245).

Nornuciferine (**22**) and xylopine (**28**) are significantly active against *Leishmania mexicana* and *Leishmania panamensis*, with the latter alkaloid showing LD_{50}=3 μM, vs. *L. mexicana*, and 37-fold higher toxicity towards the parasite than towards the host cells, the macrophages (246). Dicentrine (**39**) is active against *Trypanosoma brucei brucei in vitro* with IC_{50}=3.15 μM (247). Duguetine (**76**) is moderately active against the trypomastigote form of *Trypanosoma cruzi* (IC_{50}=9.32 μM) (120).

Asimilobine (**20**), anonaine (**23**), xylopine (**28**), isolaureline (**29**), and dicentrine (**39**) are antiplasmodial at low-to-micromolar concentrations against the chloroquine-sensitive *P. falciparum* D-2 strain and the resistant W-2 strain, but under the same conditions chloroquine has 1.3 and 11.2 nM ED_{50} values against the sensitive and the resistant strains, respectively (248). Isocorydine (**41**) is moderately active *in vitro* against *P. falciparum*, with IC_{50}=37 μM, and practically noncytotoxic and inactive against *E. histolytica* (193). Oliveroline is active against *P. falciparum* at low micromolar concentrations (27).

Dicentrine (**39**) reduces the motility of *Haemonchus contortus* larvae (the large stomach worm of ruminants), with EC_{90}=6.3 μg/mL, and an oral dose of 25 mg/kg in mice reduced the worm count by 67% (249).

G. Oxoaporphines

Atherospermidine (**86**) relaxes uterine contractions induced by high K^+ and by oxytocin, with a mechanism involving Ca^{2+} entry and release from intracellular stores (250). Liriodenine (**83**), in the 10^{-7}–10^{-4} M range, relaxes rat aorta contracted with potassium chloride or norepinephrine, but in Ca^{2+}-free medium it does not inhibit the response elicited by caffeine, indicating that its vasorelaxant action is mediated by interaction with α_1 adrenergic receptors and voltage-operated calcium channels (251). Dicentrinone (**91**) was also shown to possess weak vasorelaxant activity (252). Liriodenine (**83**) appears to regulate dopamine biosynthesis in the 5–10 μM range by reducing TH gene expression and activity, and is protective against L-DOPA-induced cytotoxicity in PC12 cells (253).

At 100 μM liriodenine (**83**) inhibits platelet aggregation, particularly that elicited by ADP or collagen, and less by AA or PAF, with aggregation falling to 5.4%, 5.3%, 40.5%, and 84.1% of controls, respectively (229,252). Lanuginosine (**87**) shows similar activity to liriodenine (**83**) (254).

Liriodenine (**83**) is cytotoxic to KB, A-549, HCT-8, and L-1210 tumor cells (255,256). It is also a mutagen for *Salmonella typhimurium* TA100 (257). Chromosomal aberrations are induced by liriodenine (**83**) at 5 μg/mL (244). Liriodenine (**83**) is selectively toxic against DNA repair- and recombination-deficient yeast mutants (IC_{12}=16.7 μg/mL vs. the rad 52 mutant), a model in which lysicamine (**84**) and *O*-methylmoschatoline (**85**) are inactive. The selectivity of liriodenine (**83**) suggested that its activity might be mediated by topoisomerase inhibition (258). Topoisomerase II inhibition by liriodenine (**83**) was confirmed in CV-1 cells infected with simian virus 40 (SV40), and it was also shown that this alkaloid is not a substrate for the verapamil-sensitive drug efflux pump (a mechanism underlying drug resistance) in CV-1 cells (248). Liriodenine

(**83**) exhibits moderate antiproliferative activity versus the human breast cancer cell lines MCF-7, the doxorubicin-resistant MCF-7/ADR, and the estrogen receptor-deficient MDA-MB435 and MT-1 lines, with IC_{50}=15.6, 16.7, 16.4, and 18.2 μM, respectively (259). In another study versus MCF-7, NCI-H460, and SF-268 cell lines, IC_{50} values of 3.19, 2.38, and 2.19 μg/mL, respectively, were recorded (260). It should be pointed out that 3.19 μg/mL corresponds to 11.6 μM, in good agreement with the earlier value. In A594 human lung cancer cells, liriodenine suppresses proliferation dose- and time-dependent in the 2−20 μM range, mainly through cell cycle inhibition (G_2/M arrest) and induction of apoptosis (261). Human hepatoma cell lines bearing the wild-type p53 oncogene (Hep G2 and SK-Hep-1) have also been challenged with liriodenine (**83**), which induced cell cycle arrest in the G_1 phase and inhibited DNA synthesis, increasing the expression of p53 and inducible nitric oxide synthase, and the intracellular NO level (262).

Lysicamine (**84**) is a modest inhibitor of the proliferation of two human liver cancer cell lines (Hep G2 and Hep 2,2,15) with IC_{50}=8.4 and 3.4 μg/mL, respectively (56). Dicentrinone (**91**) showed selective antiproliferative activity against some yeast strains, but not others. When tested against recombinant human topoisomerase I it only inhibited the enzyme to a small extent, stabilizing the enzyme−DNA binary complex (239).

Apparently, the earliest recorded biological activities of liriodenine (**83**) are antibacterial and antifungal, which it shares with lysicamine (**84**) (243,263,264). When mice infected with a lethal dose of *C. albicans* were treated with liriodenine (and also its methiodide), the proliferation of the pathogen was reduced significantly (265). The moderate activity of liriodenine (**83**) and *O*-methylmoschatoline (**85**) was demonstrated again more recently against several different fungi and bacteria (266,267).

Liriodenine (**83**) was claimed to be a fairly potent growth inhibitor of *Leishmania major* and *Leishmania donovani*, showing inhibition at 3.12 μg/mL (11.3 μM) (268), although another group reported IC_{50}=26.16 μM for a possibly different strain of *L. donovani* (269). A more recent study using *Leishmania brasiliensis* and *Leishmania guyanensis* promastigotes gave IC_{50}=58.5 and 21.5 μM, respectively, with *O*-methylmoschatoline (**85**) being about five times less active (270). Lysicamine (**84**) is also active against *L. mexicana* (245). Dicentrinone (**91**) is reported to have unusually potent leishmanicidal activity (IC_{50}=0.01 μM) (240). *O*-Methylmoschatoline inhibits the growth of *Trypanosoma brucei* at 6.25 μg/mL (268). Liriodenine (**83**) is active against *P. falciparum* with IC_{50}=15 μM (269,271).

H. Aminoethylphenanthrenes

Atherosperminine (**94**) produces behavioral stereotypy, increased spontaneous motor activity and amphetamine toxicity, reversal of

haloperidol-induced catalepsy, inhibition of conditioned avoidance response, inhibition of morphine analgesia, and potentiation of the anticonvulsant action of diphenylhydantoin, effects associated with dopamine receptor stimulation (272). It also inhibits the contraction of guinea pig trachealis muscle elicited by carbachol, prostaglandin $F_{2\alpha}$, a synthetic thromboxane analogue and leukotriene C4, it potentiates tracheal relaxation and cAMP accumulation elicited by forskolin and, at higher concentrations, by itself raises the content of cAMP, but not cGMP, in the tissue. Thus, its major mechanism of action seems to be the inhibition of cAMP phosphodiesterase (273).

At 100 μg/mL, atherosperminine (**94**) and its *N*-methyl quaternary salt completely inhibited platelet aggregation elicited by ADP, AA, collagen, or PAF, while atherosperminine *N*-oxide (**95**), though inhibiting AA- and collagen-induced aggregation, is less effective against aggregation elicited by ADP or PAF. At this dose, atherosperminine (**94**) and its *N*-oxide are also complete antagonists of high potassium or norepinephrine-induced contractions of rat thoracic aorta, pointing to simultaneous α_1-adrenoceptor and calcium channel inhibition (274).

I. Copyrine Alkaloids

Sampangine (**97**) potently inhibits HL-60 human leukemia cell proliferation by 50% at IC_{50}=2.65 μM, and its (lethal) DC_{50} value is 24.5 μM, suggesting that apoptosis plays a role in the cytotoxicity of this alkaloid, as confirmed by its effect at 20 μM on caspase-3 activity. At 4.0 μM sampangine induces cell cycle arrest in the G_0/G_1 phase, and at 20 μM leads to accumulation of cells with decreased DNA, typical of apoptotic cells. Low and high concentrations of sampangine (**97**) caused opposite effects on the potential of mitochondrial membranes, leading first to hyperpolarization (275). Treatment of HL-60 cells with sampangine (**97**) induced the rapid formation of reactive oxygen species, and quenching these with antioxidants abolished the pro-apoptotic activity of the alkaloid, indicating that sampangine-induced oxidative stress plays a key role in DNA damage (276). Sampangine (**97**) strongly inhibits the proliferation of human malignant melanoma cells (SK-MEL) with IC_{50}=0.37 μg/mL but, as observed previously in the HL-60 model, it is at least ten times less potent than other human cancer cells in culture (KB, BT-549, and SK-OV-3) (16).

Hadranthine A (**99**) was practically inactive against the human cancer cells tested, but hadranthine B (**100**) inhibited the proliferation of SK-MEL, KB, BT-549, and SK-OV-3 cells with IC_{50}=3.0, 6.4, 6.6, and 3.6 μg/mL, respectively. Imbiline-1 (**101**) inhibited SK-MEL and SK-OV-3 cells with IC_{50}=2.0 and 5.0 μg/mL, respectively, but showed IC_{50} values greater than 10 μg/mL in the other cell lines (16).

Sampangine (**97**) and 3-methoxysampangine (**98**) exhibit antifungal and antimycobacterial potencies about one half of those of amphotericin B and rifampicin, with MIC in the 0.78−1.56 μg/mL range against *C. albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, and *Mycobacterium intracellulare*, somewhat higher than the data published previously by these authors for the 3-methoxy derivative (277,278). In *Saccharomyces cerevisiae*, sampangine (**97**) induces oxidative stress, and its antifungal activity is at least partially due to alterations in heme metabolism (279).

Sampangine (**97**), 3-methoxysampangine (**98**), hadranthine A (**99**), and imbiline-1 (**101**), but not hadranthine B (**100**), exhibit antiplasmodial activity *in vitro* against *P. falciparum* (chloroquine-resistant clone W-2 and chloroquine-sensitive clone D-6). Although about ten times less potent than chloroquine against the D-6 clone, hadranthine A (**99**) shows reasonably good selectivity (selectivity index >40) versus Vero cells, while the other alkaloids are even less potent and less selective. On the other hand, sampangine (**97**) and 3-methoxysampangine (**98**) are more potent than chloroquine against the chloroquine-resistant W-2 clone (16).

J. 1-Aza-9,10-anthraquinones

Cleistopholine (**104**) inhibits the proliferation of Hep G2 and Hep 2,2,15 human hepatocarcinoma cell lines, with IC_{50}=0.22 and 0.54 μg/mL, respectively (56). It has modest antifungal and antimycobacterial activities with MIC against *C. albicans*, *C. neoformans*, *A. fumigatus*, and *M. intracellulare* of 12.5, 1.56, 100, and 12.5 μg/mL, respectively (277), and has also shown activity against mutant *S. cerevisiae* strains, *Cladosporium cladosporioides*, and *Cladosporium sphaerospermum* (54). Cleistopholine (**104**) inhibits the growth of *P. falciparum* at low micromolar concentrations (27).

VII. CONCLUDING REMARKS

The foregoing sections illustrate a cyclic trend that has been developing for a long time in natural products research, but which seems to take on specific features in studies on plant families that are traditionally seen as rich sources of alkaloids. In its initial century, from the isolation of morphine and quinine through mescaline, alkaloid chemistry was largely motivated by the desire to understand and to better apply the medicinal or biological properties of plant drugs. Later on, rapid advances in structure elucidation methodology and instrumentation led to an approach akin to the mountaineer's ''Why climb it? Because it's there!,'' while biosynthetic work remained more concerned with a quest for explanations. Over the last few decades a renewed interest in practical uses fired the development of bioassay-guided fractionation and a

preference for biosynthetic studies related to commercially or medically important alkaloids. In the meantime, organic and medicinal chemists developed synthetic methodology, and used alkaloid structural templates to generate new drugs, and fruitful collaborative efforts continue, both in the pharmaceutical industry and in academia.

In the specific case of *Duguetia*, the identification of known alkaloids and the discovery of new structures have slowed considerably, while the pharmacology of some of the more widespread constituents has made surprising progress. But something seems to be lacking. It is most likely that there is an enormous wealth of ethnopharmacological knowledge risking oblivion and still waiting to be recorded. If alkaloid chemistry is to contribute to our understanding of the biology of the genus, it needs to address a wider range of species, particularly those belonging to unexplored or little-explored sections, and metabolic profiling should be applied to many of the plants that have already been studied as well as those that have not. Bioassay-guided fractionation has yielded some spectacular results, but what bioassays should be used? Easy antibacterial assays (unlike antifungal or antiparasitic assays) do not seem to have uncovered anything of interest in higher plants, and natural products chemists are not usually qualified to identify apparently arcane biological targets such as some of those now pursued by the pharmaceutical industry, or to set up the necessary tests, stressing the need to collaborate with pharmacologists. Although much is known about the pharmacology of some *Duguetia* alkaloids commonly found in other plants, the more characteristic alkaloids such as the 7-oxygenated and the 9,11-dioxygenated aporphinoids remain practically untouched. And what about structural modification or analog synthesis?

It is hoped that these comments will stimulate discussion in the alkaloid chemical community and invigorate research, leading to both qualitative and quantitative leaps in productivity, and to novel approaches that will surely have unsuspected, but doubtless very valuable, results.

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REFERENCES

[1] The Angiosperm Phylogeny Group., *Bot. J. Linn. Soc.* **141**, 399 (2003).
[2] Y.-L. Qiu, O. Dombrowska, J. Lee, L.-B. Li, B. A. Whitlock, F. Bernasconi-Quadroni, J.S. Rest, C. C. Davis, T. Borsch, W. W. Hilu, S. S. Renner, D. E. Soltis, P. S. Soltis,

M. J. Chase, J. J. Cannone, R. R. Gutell, M. Powell, V. Savolainen, L. W. Chatrou, and M. W. Chase, *Int. J. Plant Sci.* **166**, 815 (2005).
[3] D. K. Liscombe, B. P. MacLeod, N. Loukanina, O. I. Nandi, and P. J. Facchini, *Phytochemistry* **66**, 2501 (2005).
[4] R. E. Fries, *in:* ''Die natürlichen Pflanzenfamilien'' (A. Engler and K. Prantl, eds.), 2nd Edition, vol., 17 all. Duncker & Humblot, Berlin, 1959.
[5] A. K. van Setten and J. Koek-Noorman, *Biblioth. Bot.* **142**, 1 (1992).
[6] L. W. Chatrou, J. Koek-Noorman, and P. J. M. Maas, *Ann. Missouri Bot. Gard.* **87**, 234 (2000).
[7] R. E. Fries, *Acta Horti. Berg.* **12**, 1 (1934).
[8] R. E. Fries, *Acta Horti. Berg.* **12**, 289 (1939).
[9] C. M. van Zuilen, J. Koek-Noorman, and P. J. M. Maas, *Plant Syst. Evol.* **194**, 173 (1995).
[10] C. A. Carollo, A. R. Hellmann-Carollo, J. M. de Siqueira, and S. Albuquerque, *J. Chil. Chem. Soc.* **51**, 837 (2006).
[11] D. B. da Silva, E. C. O. Tulli, W. S. Garcez, E. A. Nascimento, and J. M. de Siqueira, *J. Braz. Chem. Soc.* **18**, 1560 (2007).
[12] C. A. Carollo, J. M. de Siqueira, W. S. Garcez, R. Diniz, and N. G. Fernandes, *J. Nat. Prod.* **69**, 1222 (2006).
[13] C. A. Carollo, J. M. de Siqueira, and M. Joao, *Nat. Prod. Res.* **23**, 633 (2009).
[14] H. C. Brastianos, C. M. Sturgeon, M. Roberge, and R. J. Andersen, *J. Nat. Prod.* **70**, 287 (2007).
[15] P. P. Diaz, A. M. P. de Diaz, and P. Joseph-Nathan, *Rev. Latinoamer. Quím.* **16**, 110 (1985).
[16] I. Muhammad, D. C. Dunbar, S. Takamatsu, L. A. Walker, and A. M. Clark, *J. Nat. Prod.* **64**, 559 (2001).
[17] F. Roblot, R. Hocquemiller, H. Jacquemin, and A. Cavé, *Plantes Méd. Phytother.* **12**, 259 (1978).
[18] M. Lebœuf, F. Bévalot, and A. Cavé, *Planta Med.* **38**, 33 (1980).
[19] F. Roblot, R. Hocquemiller, and A. Cavé, *C. R. Acad. Sci. Paris, Sér. II* **293**, 373 (1981).
[20] F. Roblot, R. Hocquemiller, A. Cavé, and C. Moretti, *J. Nat. Prod.* **46**, 862 (1983).
[21] D. Debourges, F. Roblot, R. Hocquemiller, and A. Cavé, *J. Nat. Prod.* **50**, 664 (1987).
[22] D. Debourges, F. Roblot, R. Hocquemiller, and A. Cavé, *J. Nat. Prod.* **50**, 852 (1987).
[23] D. Debourges, R. Hocquemiller, and A. Cavé, *J. Nat. Prod.* **48**, 310 (1985).
[24] S. Rasamizafy, R. Hocquemiller, A. Cavé, and A. Fournet, *J. Nat. Prod.* **50**, 674 (1987).
[25] O. Arango, E. G. Pérez, H. Granados, B. Rojano, and J. Sáez, *Actual. Biol.* **26**, 105 (2005).
[26] E. G. Pérez, J. Sáez, and B. K. Cassels, *J. Chil. Chem. Soc.* **50**, 553 (2005).
[27] E. G. Pérez, J. Sáez, S. Blair, X. Franck, and B. Figadère, *Lett. Org. Chem.* **1**, 102 (2004).
[28] O. R. Gottlieb, A. F. Magalhães, E. G. Magalhães, J. G. S. Maia, and A. J. Marsaioli, *Phytochemistry* **17**, 837 (1978).
[29] I. M. Fechine, V. R. Navarro, E. V. L. da Cunha, M. S. Silva, J. G. S. Maia, and J. M. Barbosa-Filho, *Biochem. Syst. Ecol.* **30**, 267 (2002).
[30] V. R. Navarro, I. M. F. Sette, E. V. L. da Cunha, M. S. Silva, J. M. Barbosa-Filho, and J. G. S. Maia, *Rev. Bras. Pl. Med.* **3**, 23 (2001).
[31] A. A. Sáez-Vega, Ph.D. Thesis, Universidad de Antioquia, Medellín, Colombia, 2009.
[32] J. R. G. da Silva, A. S. S. Carneiro, J. M. Barbosa, M. D. Agra, M. S. da Silva, E. V. L. da Cunha, D. E. de Andrade, and R. Braz-Filho, *Biochem. Syst. Ecol.* **35**, 456 (2007).
[33] J. M. de Siqueira, M. G. Ziminiani, U. M. Resende, and M. A. D. Boaventura, *Quim. Nova* **24**, 185 (2001).
[34] D. F. Silva, D. L. Porto, I. G. Araújo, K. L. Dias, K. V. Cavalcante, R. C. Veras, J. F. Tavares, N. A. Correia, D. N. Guedes, M. S. Silva, and I. A. Medeiros, *Pharmazie* **64**, 327 (2009).

[35] I. M. Fechine, M. A. Lima, V. R. Navarro, E. V. L. Cunha, M. S. Silva, J. M. Barbosa-Filho, and J. G. S. Maia, *Rev. Bras. Farmacogn.* **12**, 17 (2002).
[36] C. Casagrande and F. Ferrari, *Farmaco, Ed. Sci.* **25**, 42 (1970).
[37] F. Bévalot, M. Lebœuf, A. Bouquet, and A. Cavé, *Ann. Pharm. Fr.* **35**, 65 (1977).
[38] H. Mathouet, A. Elomri, P. Lameiras, A. Daïch, and P. Vérité, *Phytochemistry* **68**, 1813 (2007).
[39] F. Bévalot, M. Lebœuf, and A. Cavé, *Plantes Méd. Phytother.* **11**, 315 (1977).
[40] K. Sarpong, D. K. Santra, G. J. Kapadia, and J. W. Wheeleer, *Lloydia* **40**, 616 (1977).
[41] M. D. Pirie, L. W. Chatrou, R. H. J. Erkens, J. W. Maas, T. van der Niet, J. B. Mols, and J. E. Richardson, *in:* ''Plant Species-Level Systematics'' (F. T. Bakker, L. W. Chatrou, B. Gravendeel and P. B. Pelser, eds.), vol. 143, p. 149. A. R. G. Gantner Verlag, Liechtenstein, 2005.
[42] D. L. Dilcher and T. A. Lott, *Bull. Flor. Mus. Nat. Hist.* **45**, 1 (2005).
[43] N. Wikström, V. Savolainen, and M. W. Chase, *Proc. R. Soc. Lond. B* **268**, 2211 (2001).
[44] N. Kumar and R. B. Embley, *Geol. Soc. Am. Bull.* **88**, 683 (1977).
[45] J. M. O'Connor and R. A. Duncan, *J. Geophys. Res.* **95**, 475 (1990).
[46] J. E. Richardson, L. W. Chatrou, J. B. Mols, R. H. J. Erkens, and M. D. Pirie, *Proc. R. Soc. Lond. B* **359**, 1495 (2004).
[47] F. Bévalot, M. Lebœuf, and A. Cavé, *C. R. Acad. Sci. Paris, Sér. C* **282**, 865 (1976).
[48] A. Cavé, N. Kunesch, M. Lebœuf, F. Bévalot, A. Chiaroni, and C. Riche, *J. Nat. Prod.* **43**, 103 (1980).
[49] S. Rasamizafy, R. Hocquemiller, A. Cavé, and H. Jacquemin, *J. Nat. Prod.* **49**, 1078 (1986).
[50] R. Hocquemiller, C. Debitus, F. Roblot, A. Cavé, and H. Jacquemin, *J. Nat. Prod.* **47**, 353 (1984).
[51] A. Cavé, M. Lebœuf, and B. K. Cassels, *in:* ''The Alkaloids'' (A. Brossi, ed.), vol. 35, p. 1. Academic Press, New York, 1989.
[52] Y.-C. Wu, F.-R. Chang, and C.-Y. Chen, *J. Nat. Prod.* **68**, 406 (2005).
[53] S. V. Bailleul and P. G. Waterman, *J. Nat. Prod.* **63**, 6 (2000).
[54] J. H. G. Lago, M. H. Chaves, M. C. C. Ayres, D. G. Agripino, and M. C. Young, *Planta Med.* **73**, 292 (2007).
[55] I. M. Fechine, J. F. Tavares, M. S. da Silva, J. M. Barbosa, M. de F. Agra, and E. V. L. da-Cunha, *Fitoterapia* **74**, 29 (2003).
[56] T.-J. Hsieh, F.-R. Chang, Y.-C. Chia, C.-Y. Chen, H.-F. Chin, and Y.-C. Wu, *J. Nat. Prod.* **64**, 616 (2001).
[57] A. Chiaroni, C. Riche, F. Roblot, R. Hocquemiller, and A. Cavé, *Acta Cryst.* **C39**, 1311 (1983).
[58] H. Hara, S. Komoriya, T. Miyashita, and O. Hoshino, *Tetrahedron Asymm.* **6**, 1683 (1995).
[59] I. Stepanov, S. S. Hecht, S. Ramakrishnan, and P. C. Gupta, *Int. J. Cancer* **116**, 16 (2005).
[60] T. Ohwada, M. Miura, H. Tanaka, S. Sakamoto, K. Yamaguchi, H. Ikeda, and S. Inagaki, *J. Am. Chem. Soc.* **123**, 10164 (2001).
[61] A. Sinios, *Münch. Med. Wochenschr.* **106**, 1180 (1964).
[62] A. Partheil, *Arch. Pharm.* **232**, 161 (1894).
[63] G. R. Lenz and F. J. Koszyk, *J. Chem. Soc., Perkin Trans. 1*, 1273 (1984).
[64] Y. Kitahara, M. Mochii, M. Mori, and A. Kubo, *Tetrahedron* **59**, 2885 (2003).
[65] Z.-W. Wang, W.-W. Ma, J. L. McLaughlin, and M. P. Gupta, *J. Nat. Prod.* **51**, 382 (1988).
[66] K. Hirata, C. Poeaknapo, J. Schmidt, and M. H. Zenk, *Phytochemistry* **65**, 1039 (2004).
[67] V. Mahiou, F. Roblot, A. Fournet, and R. Hocquemiller, *Phytochemistry* **54**, 709 (2000).
[68] L. W. Chatrou, *Am. J. Bot.* **84**, 861 (1997).
[69] R. Stadler and M. H. Zenk, *J. Biol. Chem.* **268**, 823 (1993).

[70] A. Winkler, F. Hartner, T. M. Kutchan, A. Glieder, and P. Macheroux, *J. Biol. Chem.* **281**, 21276 (2006).
[71] H. Böhm and E. Rink, *Biochem. Physiol. Pflanz.* **168**, 69 (1975).
[72] P. Steffens, N. Nagakura, and M. H. Zenk, *Tetrahedron Lett.* **25**, 951 (1984).
[73] A. Winkler, A. Lyskowski, S. Riedl, M. Puhl, T. M. Kutchan, P. Macheroux, and K. Gruber, *Nat. Chem. Biol.* **4**, 719 (2008).
[74] A. Winkler, M. Puhl, H. Weber, T. M. Kutchan, K. Gruber, and P. Macheroux, *Phytochemistry* **70**, 1092 (2009).
[75] M. Amann, N. Nagakura, and M. H. Zenk, *Eur. J. Biochem.* **175**, 17 (1988).
[76] E. Galneder, M. Rüffer, G. Wanner, M. Tabata, and M. H. Zenk, *Plant Cell Rep.* **7**, 1 (1988).
[77] T. M. Kutchan and H. Dittrich, *J. Biol. Chem.* **270**, 24475 (1995).
[78] W. Cui, K. Iwasa, M. Sugiura, A. Takeuchi, C. Tode, Y. Nishiyama, M. Moriyasu, H. Tokuda, and K. Takeda, *J. Nat. Prod.* **70**, 1771 (2007).
[79] D. K. Liscombe and P. J. Facchini, *J. Biol. Chem.* **282**, 14741 (2007).
[80] M. Shamma, ''The Isoquinoline Alkaloids – Chemistry and Pharmacology'', Academic Press, New York, NY, 1972.
[81] Y.-C. Chang, F.-R. Chang, A. T. Khalil, P.-W. Hsieh, and Y.-C. Wu, *Z. Naturforsch. C* **58**, 521 (2003).
[82] N. Ikezawa, K. Iwasa, and F. Sato, *Plant Cell Rep.* **28**, 123 (2009).
[83] D. S. Bhakuni, V. K. Mangla, A. N. Singh, and R. S. Kapil, *J. Chem. Soc., Perkin Trans. 1*, 267 (1978).
[84] R. Gerardy and M. H. Zenk, *Phytochemistry* **32**, 79 (1993).
[85] N. Ikezawa, K. Iwasa, and F. Sato, *J. Biol. Chem.* **283**, 8810 (2008).
[86] C.-W. Lin, X.-F. Wang, F.-X. Zhou, X.-Y. Jiang, X.-X. Wu, and S.-K. Zhao, *Zhiwu Xuebao* **31**, 449 (1989).
[87] M. L. Cornelio, J. M. Barbosa-Filho, S. F. Cortés, and G. Thomas, *Planta Med.* **65**, 462 (1999).
[88] R. Hocquemiller, C. Debitus, F. Roblot, and A. Cavé, *Tetrahedron Lett.* **23**, 4247 (1982).
[89] N. Murugesan and M. Shamma, *Tetrahedron Lett.* **20**, 4251 (1979).
[90] C.-M. Chiou, J.-J. Kang, and S.-S. Lee, *J. Nat. Prod.* **61**, 46 (1998).
[91] A. Cavé, M. Lebœuf, and P. G. Waterman, *in:* ''Alkaloids: Chemical and Biological Perspectives'' (S. W. Pelletier, ed.), vol. 5, p. 133. Wiley, New York, 1987.
[92] W. C. Taylor, *Aust. J. Chem.* **37**, 1095 (1984).
[93] D. Tadić, B. K. Cassels, M. Lebœuf, and A. Cavé, *Phytochemistry* **26**, 537 (1987).
[94] Z.-F. Chen, Y.-C. Liu, L.-M. Liu, H.-S. Wang, S.-H. Qin, B.-L. Wang, H.-D. Bian, B. Yang, H.-K. Fun, H.-G. Liu, H. Liang, and C. Orvig, *Dalton Trans.*, 262 (2009).
[95] A. Cavé, *in:* ''The Chemistry and Biology of Isoquinoline Alkaloids'' (J. D. Phillipson, M. F. Roberts and M. H. Zenk, eds.), Springer-Verlag, Berlin, 1985.
[96] D. Cortes, A. Ramahatra, A. Cavé, J. de C. Bayma, and H. Dadoun, *J. Nat. Prod.* **48**, 254 (1985).
[97] L. Castedo, J. A. Granja, A. Rodríguez de Lera, and M. C. Villaverde, *J. Heterocycl. Chem.* **25**, 1561 (1988).
[98] M. S. El Tohami, Ph.D. Thesis, Université de Paris-Sud, Châtenay-Malabry, France, 1984.
[99] R. H. J. Erkens, L. W. Chatrou, J. W. Maas, T. van der Niet, and V. Savolainen, *Mol. Phylogen. Evol.* **44**, 399 (2007).
[100] B. K. Cassels, A. Cavé, D. Davoust, R. Hocquemiller, S. Rasamizafy, and D. Tadić, *J. Chem. Soc., Chem. Commun.* **1481** (1986).
[101] W. Eisenreich and A. Bacher, *Phytochemistry* **68**, 2799 (2007).
[102] K. Dettmer, P. A. Aronov, and B. D. Hammock, *Mass Spectrom. Rev.* **26**, 51 (2007).

[103] J. Ribeiro, *in* ''Flora da reserva Ducke: Guia de identificação das plantas vasculares de una floresta de terra firme na Amazónia Central'', p. 816. INPA, Manaos, Brasil, 1999.
[104] M. F. C. Matos, L. I. S. P. Leite, D. Brustolim, J. M. de Siqueira, A. M. Carollo, A. R. Hellmann, N. F. G. Pereira, and D. B. da Silva, *Fitoterapia* **77**, 227 (2006).
[105] Ynca Garcilasso de la Vega, ''Primera Parte de los Commentarios Reales ...'', P. Craesbeeck, Lisbon, 1609.
[106] J. W. Banning, N. J. Uretsky, P. N. Patil, and J. L. Beal, *Life Sci.* **26**, 2083 (1980).
[107] H. Watanabe, M. Ikeda, K. Watanabe, and T. Kikuchi, *Planta Med.* **42**, 213 (1981).
[108] H. Watanabe, K. Watanabe, and T. Kikuchi, *J. Pharmacobiodyn.* **6**, 793 (1983).
[109] L. C. Morais, J. M. Barbosa-Filho, and R. N. Almeida, *J. Ethnopharmacol.* **62**, 57 (1998).
[110] A. Lannuzel, P. P. Michel, D. Caparros-Lefebvre, J. Abaul, and R. Hocquemiller, *Mov. Disord.* **17**, 84 (2002).
[111] I. Kimura, M. Kimura, M. Yoshizaki, K. Yanada, S. Kadota, and T. Kikuchi, *Planta Med.* **48**, 43 (1983).
[112] I. Kimura, L. H. Chui, K. Fujitani, T. Kikuchi, and M. Kimura, *Jpn. J. Pharmacol.* **50**, 75 (1989).
[113] M. L. Martin, M. T. Diaz, M. J. Montero, P. Prieto, L. San Roman, and D. Cortes, *Planta Med.* **59**, 63 (1993).
[114] M. A. Medeiros, X. P. Nunes, J. M. Barbosa-Filho, V. S. Lemos, J. F. Pinho, D. Roman-Campos, I. A. de Medeiros, D. A. Araújo, and J. S. Cruz, *Naunyn-Schmiedebergs Arch. Pharmacol.* **379**, 115 (2009).
[115] K. L. Dias, C. da S. Dias, J. M. Barbosa-Filho, R. N. Almeida, N. de A. Correia, and I. A. Medeiros, *Planta Med.* **70**, 328 (2004).
[116] J.-J. Chen, Y.-L. Chang, C.-M. Teng, and I.-S. Chen, *Planta Med.* **66**, 251 (2000).
[117] M. de Q. Paulo, J. M. Barbosa-Filho, E. Q. Lima, R. F. Maia, R. de C. Barbosa, and M. A. Kaplan, *J. Ethnopharmacol.* **36**, 39 (1992).
[118] K. Likhitwitayawuid, C. K. Angerhofer, H. Chai, J. M. Pezzuto, and G. A. Cordell, *J. Nat. Prod.* **56**, 1468 (1993).
[119] K. Nakaoji, H. Nayeshiro, and T. Tanahashi, *Biol. Pharm. Bull.* **20**, 586 (1997).
[120] Q. Zhao, Y. Zhao, and K. Wang, *J. Ethnopharmacol.* **106**, 408 (2006).
[121] A. Granjon and A. M. Beau, *Bull. Féd. Soc. Gynécol. Obstét. Lang. Fr.* **3**, 63 (1951).
[122] P. Gley, R. Rothstein, and J. Delor, *C. R. Séances Soc. Biol. Fil.* **146**, 13 (1952).
[123] L. Mambu, M. T. Martin, D. Razafimahefa, D. Ramanitrahasimbola, P. Rasoanaivo, and F. Frappier, *Planta Med.* **66**, 537 (2000).
[124] A. L. Otshudi, S. Apers, L. Pieters, M. Claeys, C. Pannecouque, E. De Clercq, A. Van Zeebroeck, S. Lauwers, M. Frédérich, and A. Foriers, *J. Ethnopharmacol.* **102**, 89 (2005).
[125] F.-N. Ko, S.-M. Yu, M.-J. Su, Y.-C. Wu, and C.-M. Teng, *Br. J. Pharmacol.* **110**, 882 (1993).
[126] F.-N. Ko, J.-H. Guh, S.-M. Yu, Y.-S. Hou, Y.-C. Wu, and C.-M. Teng, *Br. J. Pharmacol.* **112**, 1174 (1994).
[127] J.-H. Guh, C.-H. Hsieh, and C.-M. Teng, *Eur. J. Pharmacol.* **374**, 503 (1999).
[128] Y.-C. Chia, F.-R. Chang, C.-C. Wu, C.-M. Teng, K.-S. Chen, and Y.-C. Wu, *Planta Med.* **72**, 1238 (2006).
[129] I. N. Vinokurov, B. A. Somov, Iu. S. Butov, and N. N. Ostrovskiĭ, *Vest. Dermatol. Venerol.* **44**, 78 (1970).
[130] J. Yamahara, *Nippon Yakurigaku Zasshi.* **72**, 909 (1976).
[131] G.-O. Liu, S. Algeri, and S. Garattini, *Arch. Int. Pharmacodyn. Thér.* **258**, 39 (1982).
[132] E. V. Arzamastsev, M. I. Mironova, L. V. Krepkova, V. V. Bortnikova, and Iu. V. Kuznetsov, *Farmakol. Toksikol.* **46**, 107 (1983).
[133] G.-Z. Jin, X.-L. Wang, and W.-X. Shi, *Sci. Sin. B* **29**, 527 (1986).
[134] G.-Z. Jin, S.-X. Xu, and L.-P. Yu, *Sci. Sin. B* **29**, 1054 (1986).
[135] F. Marcenac, G.-Z. Jin, and F. Gonon, *Psychopharmacology (Berl.)* **89**, 89 (1986).

[136] S.-X. Xu, L.-P. Yu, Y.-R. Han, Y. Chen, and G.-Z. Jin, *Zhongguo Yao Li Xue Bao* **10**, 104 (1989).
[137] L.-J. Chen, X. Guo, Q.-M. Wang, and G.-Z. Jin, *Zhongguo Yao Li Xue Bao* **13**, 442 (1992).
[138] Z.-Z. Ma, W. Xu, N. H. Jensen, B. L. Roth, L.-Y. Liu-Chen, and D.-Y. Lee, *Molecules* **13**, 2303 (2008).
[139] M.-T. Hsieh, W.-H. Peng, and C.-C. Hsieh, *Chin. J. Physiol.* **37**, 79 (1994).
[140] J.-Y. Hu and G.-Z. Jin, *Zhongguo Yao Li Xue Bao* **20**, 193 (1999).
[141] J.-Y. Hu and G.-Z. Jin, *Zhongguo Yao Li Xue Bao* **20**, 715 (1999).
[142] H. Chu, G. Jin, E. Friedman, and X. Zhen, *Cell. Mol. Neurobiol.* **28**, 491 (2008).
[143] X.-Z. Zhu, Z. -Zhou, X.-Q. Ji, J. Gu, L.-G. Guo, and F.-S. Wang, *Zhongguo Yao Li Xue Bao* **12**, 104 (1991).
[144] J.-Y. Hu and G.-Z. Jin, *Acta Pharmacol. Sin.* **21**, 439 (2000).
[145] F.-Y. Chueh, M.-T. Hsieh, C.-F. Chen, and M.-T. Lin, *Pharmacology* **51**, 237 (1995).
[146] F. Sun and D.-X. Li, *Zhongguo Yao Li Xue Bao* **10**, 30 (1989).
[147] G.-Q. Liu, B.-Y. Han, and E.-H. Wang, *Zhongguo Yao Li Xue Bao* **10**, 302 (1989).
[148] B. Xuan, D.-X. Li, and W. Wang, *Zhongguo Yao Li Xue Bao* **13**, 167 (1992).
[149] J. Zhou, B. Xuan, and D.-X. Li, *Zhongguo Yao Li Xue Bao* **14**, 130 (1993).
[150] D.-J. Wang, H.-Y. Mao, and M. Lei, *Zhongguo Zhong Xi Yi Jie He Za Zhi* **13**, 455 (1993).
[151] D.-J. Wang, *Zhongguo Zhong Xin Xue Guan Bing Za Zhi* **21**, 286 (1993).
[152] P. Chan, W.-T. Chiu, Y.-J. Chen, P.-J. Wu, and J.-T. Cheng, *Planta Med.* **65**, 340 (1999).
[153] F.-Y. Chuen, M.-T. Hsieh, C.-F. Chen, and M.-T. Lin, *Jpn. J. Pharmacol.* **69**, 177 (1995).
[154] M.-T. Lin, F.-Y. Chueh, M.-T. Hsieh, and C.-F. Chen, *Clin. Exp. Pharmacol. Physiol.* **23**, 738 (1996).
[155] M.-T. Lin, F.-Y. Chueh, and M.-T. Hsieh, *Neurosci. Lett.* **23**, 315 (2001).
[156] C.-K. Chang and M.-T. Lin, *Neurosci. Lett.* **307**, 163 (2001).
[157] M.-T. Lin, J.-J. Wang, and M.-S. Young, *Neurosci. Lett.* **320**, 113 (2002).
[158] C. Halbsguth, O. Meissner, and H. Häberlein, *Planta Med.* **69**, 305 (2003).
[159] W.-C. Leung, H. Zheng, M. Huen, S.-L. Law, and H. Xue, *Prog. Neuropsychopharmacol. Biol. Psychiatry* **27**, 775 (2003).
[160] Y.-H. Ren, Y. Zhu, G.-Z. Jin, and J.-W. Zheng, *Chin. J. Drug. Depend.* **9**, 182 (2000).
[161] J.-Y. Luo, Y.-H. Ren, R. Zhu, D.-Q. Lin, and J.-W. Zheng, *Chin. J. Drug. Depend.* **12**, 177 (2003).
[162] J. R. Mantsch, S.-J. Li, R. Risinger, S. Awad, E. Katz, D. A. Baker, and Z. Yang, *Psychopharmacology (Berl.)* **192**, 581 (2007).
[163] Z.-X. Xi, Z. Yang, S.-J. Li, X. Li, C. Dillon, X.-Q. Peng, K. Spiller, and E. L. Gardner, *Neuropharmacology* **53**, 771 (2007).
[164] Y.-L. Liu, J.-H. Liang, L.-D. Yan, R.-B. Su, C.-F. Wu, and Z.-H. Gong, *Acta Pharmacol. Sin.* **26**, 533 (2005).
[165] Y.-L. Liu, L.-D. Yan, P.-L. Zhou, C.-F. Wu, and G.-H. Gong, *Eur. J. Pharmacol.* **602**, 321 (2008).
[166] M. W. Schewe, *Acta Biol. Med. Ger.* **35**, 1019 (1976).
[167] X. Su, F. Qin, L. Kong, J. Ou, C. Xie, and H. Zou, *J. Chromatogr. B* **845**, 174 (2007).
[168] S.-S. Bun, M. Laget, A. Chea, H. Bun, E. Ollivier, and R. Elias, *Phytother. Res.* **23**, 587 (2009).
[169] M.-T. Hsieh and L.-Y. Wu, *J. Pharm. Pharmacol.* **48**, 959 (1996).
[170] C.-K. Chang, F.-Y. Chueh, M.-T. Hsieh, and M.-T. Lin, *Neurosci. Lett.* **28**, 109 (1999).
[171] G. Yang, P. Wang, Y. Tang, C. Jiang, and D. Wang, *J. Tongji Med. Univ.* **19**, 285 (1999).
[172] G. Yang, C. Jiang, Y. Tang, and D. Wang, *J. Tongji Med. Univ.* **20**, 106 (2000).
[173] B. Liu and G. Yang, *J. Huazhong Univ. Sci. Technolog. Med. Sci.* **24**, 445 (2004).
[174] Z.-M. Zhang, X.-X. Zheng, B. Jiang, and Q. Zhou, *Zhongguo Zhong Yao Za Zhi* **29**, 371 (2004).
[175] Z.-M. Zhang, B. Jiang, and X.-X. Zheng, *Zhongguo Zhong Yao Za Zhi* **30**, 861 (2005).

[176] T.-B. Ng, F. Liu, and Z.-T. Wang, *Life Sci.* **66**, 709 (2000).
[177] Q. Min, Y.-T. Bai, S.-J. Shu, and P. Ren, *Zhongguo Zhong Yao Za Zhi* **31**, 483 (2006).
[178] B. Das, V. Tandon, L. M. Lyndem, A. I. Gray, and V. A. Ferro, *Comp. Biochem. Physiol. C* **149**, 397 (2009).
[179] N. T. Nguyen, V. C. Pham, M. Litaudon, F. Guéritte, P. Grellier, V. T. Nguyen, and V. H. Nguyen, *J. Nat. Prod.* **71**, 2057 (2008).
[180] A. Chea, S. Hout, S.-S. Bun, N. Tabatadze, M. Gasquet, N. Azas, R. Elias, and G. Balansard, *J. Ethnopharmacol.* **112**, 132 (2007).
[181] W.-N. Wu, J. L. Beal, L. A. Mitscher, K. N. Salman, and P. Patil, *Lloydia* **39**, 204 (1976).
[182] H. Han and D.-C. Fang, *Zhongguo Yao Li Xue Bao* **10**, 385 (1989).
[183] L.-D. Kong, C.-H. Cheng, and R.-X. Tan, *Planta Med.* **67**, 74 (2001).
[184] Y. Fu, B. Hu, Q. Tang, Q. Fu, and J. Xiang, *J. Huazhong Univ. Sci. Technolog. Med. Sci.* **25**, 491 (2005).
[185] K. Ingkaninan, P. Phengpa, S. Yuenyongsawad, and N. Khorana, *J. Pharm. Pharmacol.* **58**, 695 (2006).
[186] A. Volleková, D. Košťálová, V. Kettmann, and J. Tóth, *Phytother. Res.* **17**, 834 (2003).
[187] L. Slobodníková, D. Košťálová, D. Labudová, D. Kotulová, and V. Kettmann, *Phytother. Res.* **18**, 674 (2004).
[188] J. L. Vennerstrom and D. L. Klayman, *J. Med. Chem.* **31**, 1084 (1988).
[189] C. W. Wright, S. J. Marshall, P. F. Russell, M. M. Anderson, J. D. Phillipson, G. C. Kirby, D. C. Warhurst, and P. L. Schiff Jr., *J. Nat. Prod.* **63**, 1638 (2000).
[190] H. Subeki, K. Matsuura, M. Takahashi, M. Yamasaki, O. Yamato, Y. Maede, K. Katakura, M. Suzuki, T. Chairul, and T. Yoshihara, *Vet. Med. Sci.* **67**, 223 (2005).
[191] M. Vicková, V. Kubán, J. Vicar, and V. Simánek, *Electrophoresis* **26**, 1673 (2005).
[192] X. Su, L. Kong, X. Li, X. Chen, M. Guo, and H. Zou, *J. Chromatogr. A* **1076**, 118 (2005).
[193] W.-H. Chen, C.-L. Chan, Z. Cai, G.-A. Luo, and Z.-H. Jiang, *Bioorg. Med. Chem. Lett.* **14**, 4955 (2004).
[194] W.-H. Chen, Y. Qin, Z. Cai, C.-L. Chan, G.-A. Luo, and Z.-H. Jiang, *Bioorg. Med. Chem.* **13**, 1859 (2005).
[195] Y. Qin, W.-H. Chen, J.-Y. Pang, Z.-Z. Zhao, L. Liu, and Z.-H. Jiang, *Chem. Biodivers.* **4**, 145 (2007).
[196] M. Černáková, D. Košťálová, V. Kettmann, M. Plodová, J. Tóth, and J. Drimal, *BMC Complement. Altern. Med.* **19**, 2 (2002).
[197] L. Račková, M. Májeková, D. Košťálová, and M. Štefek, *Bioorg. Med. Chem.* **12**, 4709 (2004).
[198] Y. Hu, X. Chen, H. Duan, Y. Hu, and X. Mu, *Cell Biochem. Funct.* **27**, 284 (2009).
[199] Y. Hu, X. Chen, H. Duan, Y. Hu, and X. Mu, *Immunopharmacol. Immunotoxicol.* **31**, 500 (2009).
[200] Y. Li, W. He, J. Liu, F. Sheng, Z. Hu, and X. Chen, *Biochim. Biophys. Acta* **1722**, 15 (2005).
[201] B. Buffa, G. Costa, and P. Ghirardi, *Curr. Ther. Res., Clin. Exper.* **16**, 621 (1974).
[202] A. Marzo, P. Ghirardi, C. Casagrande, G. Catenazzo, and O. Mantero, *Eur. J. Clin. Pharmacol.* **13**, 219 (1978).
[203] M. Chaumontet, M. Capt, and P. Gold-Aubert, *Arzneimittelforschung* **28**, 2119 (1978).
[204] M. Galeone, D. Cacioli, G. Moise, G. Gherardi, and G. Quadro, *Curr. Ther. Res., Clin. Exper.* **30**, 44 (1981).
[205] P. Cheng, Y.-B. Ma, S.-Y. Yao, Q. Zhang, E.-J. Wang, M.-H. Yan, X.-M. Zhang, F.-X. Zhang, and J.-J. Chen, *Bioorg. Med. Chem. Lett.* **17**, 5316 (2007).
[206] G. A. Cordell, M. L. Quinn-Beattie, and N. R. Farnsworth, *Phytother. Res.* **15**, 183 (2001).
[207] S. Chuliá, M. D. Ivorra, A. Cavé, D. Cortés, M. A. Noguera, and M. P. D'Ocón, *J. Pharm. Pharmacol.* **47**, 647 (1995).
[208] M. Valiente, P. D'Ocon, M. A. Noguera, B. K. Cassels, C. Lugnier, and M. D. Ivorra, *Planta Med.* **70**, 603 (2004).

[209] G.-Q. Li, B.-Y. Han, and E.-H. Wang, *Zhongguo Yao Li Xue Bao* **10**, 302 (1989).
[210] L. Yang, Y.-X. Xu, and G.-S. Zhao, *Yao Xue Xue Bao* **25**, 859 (1990).
[211] B. Sotniková, V. Kettmann, D. Košťálová, and E. Táborská, *Methods Find. Exp. Clin. Pharmacol.* **19**, 589 (1997).
[212] Y.-Q. Zhao, G.-R. Li, D.-Z. Zhang, and G.-S. Zhao, *Zhongguo Yao Li Xue Bao* **12**, 324 (1991).
[213] N. Shoji, A. Umeyama, N. Saito, A. Iuchi, T. Takemoto, A. Kajiwara, and Y. Ohizumi, *J. Nat. Prod.* **50**, 773 (1987).
[214] H.-L. Li, R.-P. Zhang, H.-T. Ye, and H. Wang, *Zhongguo Zhong Yao Za Zhi* **25**, 426 (2000).
[215] J. A. Hasrat, T. De Bruyne, J. P. De Backer, G. Vauquelin, and A. J. Vlietinck, *J. Pharm. Pharmacol.* **49**, 1145 (1997).
[216] S. Gafner, B. M. Dietz, K. L. McPhail, I. M. Scott, J. A. Glinski, F. E. Russell, M. M. McCollom, J. W. Budzinski, B. C. Foster, C. Bergeron, M.-R. Rhyu, and J. L. Bolton, *J. Nat. Prod.* **69**, 432 (2006).
[217] V. S. Lemos, G. Thomas, and J. M. Barbosa-Filho, *J. Ethnopharmacol.* **40**, 141 (1993).
[218] S.-M. Yu, C.-C. Chen, F.-N. Ko, Y.-L. Huang, T.-F. Huang, and C.-M. Teng, *Biochem. Pharmacol.* **43**, 323 (1992).
[219] T.-H. Tsai, G.-J. Wang, and L.-C. Lin, *J. Nat. Prod.* **71**, 289 (2008).
[220] S.-M. Yu, S.-Y. Hsu, F.-N. Ko, C.-C. Chen, Y.-L. Huang, T.-F. Huang, and C.-M. Teng, *Br. J. Pharmacol.* **106**, 797 (1992).
[221] S.-M. Yu, Y.-F. Kang, C.-C. Chen, and C.-M. Teng, *Br. J. Pharmacol.* **108**, 1055 (1993).
[222] M.-J. Su, Y.-C. Nieh, H.-W. Huang, and C.-C. Chen, *Naunyn-Schmiedebergs. Arch. Pharmacol.* **349**, 42 (1994).
[223] M.-L. Young, M.-J. Su, M.-H. Wu, and C.-C. Chen, *Br. J. Pharmacol.* **113**, 69 (1994).
[224] K.-C. Chang, H. M. Lo, F.-Y. Lin, Y.-Z. Tseng, F.-N. Ko, and C.-M. Teng, *J. Cardiovasc. Pharmacol.* **26**, 169 (1995).
[225] T.-J. Tsai, R.-H. Lin, C.-C. Chan, C.-F. Chen, F.-N. Ko, and C.-M. Teng, *Nephron* **70**, 91 (1995).
[226] S.-M. Yu, F.-N. Ko, S.-C. Chueh, J. Chen, S.-C. Chen, C.-C. Chen, and T.-M. Teng, *Eur. J. Pharmacol.* **252**, 29 (1994).
[227] P. Protais, J. Arbaoui, E. Bakkali, A. Bermejo, and D. Cortes, *J. Nat. Prod.* **58**, 1475 (1995).
[228] C.-M. Jin, J.-J. Lee, Y.-K. Kim, S.-Y. Ryu, S.-C. Lim, B.-Y. Hwang, C.-K. Lee, and M.-K. Lee, *J. Asian Nat. Prod. Res.* **10**, 747 (2008).
[229] K.-S. Chen, F.-N. Ko, C.-M. Teng, and Y.-C. Wu, *Planta Med.* **62**, 133 (1996).
[230] C.-C. Chen, Y.-L. Huang, J.-C. Ou, M.-J. Su, S.-M. Yu, and C.-M. Teng, *Planta Med.* **57**, 406 (1991).
[231] C.-M. Teng, S.-M. Yu, F.-N. Ko, C.-C. Chen, Y.-L. Huang, and T.-F. Huang, *Br. J. Pharmacol.* **104**, 651 (1991).
[232] T.-C. Chi, S.-S. Lee, and M.-J. Su, *Planta Med.* **72**, 1175 (2006).
[233] L. A. Martínez, J. L. Ríos, M. Payá, and M. J. Alcaraz, *Free Radic. Biol. Med.* **12**, 287 (1992).
[234] E. R. Correché, S. A. Andujar, R. R. Kurdelas, M. J. Gómez Lechón, M. L. Freile, and R. D. Enriz, *Bioorg. Med. Chem.* **16**, 3641 (2008).
[235] C.-Y. Chen, T.-Z. Liu, W.-C. Tseng, F.-J. Lu, R.-P. Hung, C.-H. Chen, and C.-H. Chen, *Food Chem. Toxicol.* **46**, 2694 (2008).
[236] Y. Kondo, Y. Imai, H. Hojo, T. Endo, and S. Nozoe, *J. Pharmacobiodyn.* **13**, 426 (1990).
[237] R.-L. Huang, C.-C. Chen, Y.-L. Huang, J.-C. Ou, C.-P. Hu, C.-F. Chen, and C. Chang, *Planta Med.* **64**, 212 (1998).
[238] S.-H. Woo, N.-J. Sun, M. M. Cassady, and R. M. Snapka, *Biochem. Pharmacol.* **57**, 1141 (1999).
[239] B.-N. Zhou, R. K. Johnson, M. R. Mattern, X. Wang, S. M. Hecht, H. T. Beck, A. Ortiz, and D. S. Kingston, *J. Nat. Prod.* **63**, 217 (2000).

[240] D. B. da Silva, E. C. Tulli, G. C. Militão, L. V. Costa-Lotufo, C. Pessoa, M. O. de Moraes, S. Albuquerque, and J. M. de Siqueira, *Phytomedicine* **16**, 1059 (2009).
[241] J. A. Montanha, M. Amorors, J. Boustie, and L. Girre, *Planta Med.* **61**, 419 (1995).
[242] I.-L. Tsai, Y.-F. Liou, and S.-T. Lu, *Gaoxiong Yi Xue Ke Xue Za Zhi* **5**, 132 (1989).
[243] A. Villar, M. Mares, J. L. Ríos, E. Canton, and M. Gobernado, *Pharmazie* **42**, 248 (1987).
[244] S. Tadaki, T. Nozaka, S. Yamada, M. Ishino, I. Morimoto, A. Tanaka, and J. Kunitomo, *J. Pharmacobiodyn.* **15**, 501 (1992).
[245] K. Morteza-Semnani, Gh. Amin, M. R. Shidfar, H. Hadizadeh, and A. Shafiee, *Fitoterapia* **74**, 493 (2003).
[246] H. Montenegro, M. Gutiérrez, L. I. Romero, E. Ortega-Barría, T. L. Capson, and L. C. Ríos, *Planta Med.* **69**, 677 (2003).
[247] S. Hoet, C. Stévigny, S. Block, F. Opperdoes, P. Colson, B. Baldeyrou, A. Lansiaux, C. Bailly, and J. Quetin-Leclercq, *Planta Med.* **70**, 407 (2004).
[248] S. H. Woo, M. C. Reynolds, N. J. Sun, J. M. Cassady, and R. M. Snapka, *Biochem. Pharmacol.* **54**, 467 (1997).
[249] S. Ayers, D. L. Zink, K. Mohn, J. S. Powell, C. M. Brown, T. Murphy, R. Brand, S. Pretorius, D. Stevenson, D. Thompson, and S. B. Singh, *Planta Med.* **73**, 296 (2007).
[250] D. Cortes, M. Y. Torrero, M. P. D'Ocon, M. L. Candenas, A. Cavé, and A. H. A. Hadi, *J. Nat. Prod.* **53**, 503 (1990).
[251] S. Chuliá, M.-A. Noguera, M. D. Ivorra, D. Cortes, and M. P. D'Ocón, *Pharmacology* **50**, 380 (1995).
[252] K.-S. Chen, Y.-C. Wu, C.-M. Teng, G.-N. Ko, and T.-S. Wu, *J. Nat. Prod.* **60**, 645 (1997).
[253] C.-M. Jin, J.-J. Lee, Y.-J. Yang, Y.-M. Kim, Y.-K. Kim, S.-Y. Ryu, and M.-K. Lee, *Arch. Pharm. Res.* **30**, 984 (2007).
[254] M.-K. Pyo, J.-S. Yun-Choi, and Y.-J. Hong, *Planta Med.* **69**, 267 (2003).
[255] I. Borup-Grochtmann and D. G. I. Kingston, *J. Nat. Prod.* **45**, 102 (1982).
[256] Y.-C. Wu, T. Yamagishi, and K.-H. Lee, *Gaoxiong Yi Xue Ke Xue Za Zhi* **5**, 409 (1989).
[257] T. Nozaka, F. Watanabe, S. Tadaki, M. Ishino, I. Morimoto, J. Kunitomo, H. Ishii, and S. Natori, *Mutat. Res.* **240**, 267 (1990).
[258] G. A. Harrigan, A. A. L. Gunatilaka, and D. G. I. Kingston, *J. Nat. Prod.* **57**, 68 (1994).
[259] S. Khamis, M. C. Bibby, J. E. Brown, P. A. Cooper, I. Scowen, and C. W. Wright, *Phytother. Res.* **18**, 507 (2004).
[260] C.-H. Yang, M.-J. Cheng, S.-J. Lee, C.-W. Yang, H.-S. Chang, and I.-S. Cheng, *Chem. Biodivers.* **6**, 846 (2009).
[261] H.-C. Chang, F.-R. Chang, Y.-C. Wu, and Y.-H. Lai, *Kaohsiung J. Med. Sci.* **20**, 363 (2004).
[262] T.-J. Hsieh, T.-Z. Liu, C.-L. Chern, D.-A. Tsao, F.-J. Lu, Y.-H. Syu, P.-Y. Hsieh, H.-S. Hu, T.-T. Chang, and C.-H. Chen, *Food Chem. Toxicol.* **43**, 1117 (2005).
[263] C. D. Hufford, M. J. Funderburk, J. M. Morgan, and L. W. Robertson, *J. Pharm. Sci.* **64**, 789 (1975).
[264] C. D. Hufford, A. S. Sharma, and B. O. Oguntimein, *J. Pharm. Sci.* **69**, 1180 (1980).
[265] A. M. Clark, E. S. Watson, M. K. Ashfaq, and C. D. Hufford, *Pharm. Res.* **4**, 495 (1987).
[266] A. P. Nissanka, V. Karunaratne, B. M. Bandara, V. Kumar, T. Nakanishi, M. Nishi, A. Inada, L. M. Tillekeratne, D. S. Wijesundara, and A. A. Gunatilaka, *Phytochemistry* **56**, 857 (2001).
[267] M. M. Rahman, S. S. Lopa, G. Sadik, H. Rashid, R. Islam, P. Khondkar, A. H. M. K. Alam, and M. A. Rashid, *Fitoterapia* **76**, 758 (2005).
[268] A. I. Waechter, A. Cavé, R. Hocquemiller, C. Bories, V. Muñoz, and A. Fournet, *Phytother. Res.* **13**, 175 (1999).
[269] M. R. Camacho, G. C. Kirby, D. C. Warhurst, S. L. Croft, and J. D. Phillipson, *Planta Med.* **66**, 478 (2000).

[270] E. V. Costa, M. L. B. Pinheiro, C. M. Xavier, J. R. A. Silva, A. C. F. Amaral, A. D. L. Souza, A. Barison, F. R. Campos, A. G. Ferreira, G. M. C. Machado, and L. L. P. Leon, *J. Nat. Prod.* **69**, 292 (2006).
[271] J. A. Mbah, P. Tane, B. T. Ngadjui, J. D. Connolly, C. C. Okunji, M. M. Iwu, and B. M. Schuster, *Planta Med.* **70**, 437 (2004).
[272] S. K. Bhattacharya, R. Bose, P. Ghosh, V. J. Tripathi, A. B. Ray, and B. Dasgupta, *Psychopharmacology (Berl.)* **59**, 29 (1978).
[273] C.-H. Lin, F.-N. Ko, Y.-C. Wu, S.-T. Lu, and C.-M. Teng, *Eur. J. Pharmacol.* **237**, 109 (1993).
[274] K.-S. Chen, F.-N. Ko, C.-M. Teng, and Y.-C. Wu, *J. Nat. Prod.* **59**, 531 (1996).
[275] J. Kluza, A. M. Clark, and C. Bailly, *Ann. N. Y. Acad. Sci.* **1010**, 331 (2003).
[276] J. Kluza, R. Mazinghien, K. Degardin, A. Lansiaux, and A. M. Clark, *Eur. J. Pharmacol.* **525**, 32 (2005).
[277] J. R. Peterson, J. K. Zjawiony, S. Liu, C. D. Hufford, A. M. Clark, and R. D. Rogers, *J. Med. Chem.* **35**, 4069 (1992).
[278] S. Liu, B. Oguntimein, C. D. Hufford, and A. M. Clark, *Antimicrob. Agents Chemother.* **34**, 529 (1990).
[279] A. K. Agarwal, T. Xu, M. R. Jacob, Q. Feng, M. C. Lorenz, L. A. Walker, and A. M. Clark, *Eukaryotic Cell* **7**, 387 (1998).