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A CONVENIENT, RENEWABLE SOURCE OF THE ANXIOLYTIC PROAPORPHINE ALKALOID GLAZIOVINE: *DUGUETIA VALLICOLA* LEAVES

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ABSTRACT

The leaves of *Duguetia vallicola*, a fairly common, large evergreen tree from the coastal regions of Panama, Colombia and Venezuela, contain (6a*S*)-glaziovine (**1**) as a major constituent. The abundance and renewable character of the plant material make it an attractive source for this rare, psychoactive alkaloid. The availability of glaziovine and its *O*-methyl derivative pronuciferine (**2**) has made complete ^{1}H and ^{13}C NMR assignments of proaporphines possible for the first time.

KEYWORDS: Duguetia vallicola, *glaziovine, pronuciferine, proaporphines*

INTRODUCTION

Glaziovine (**1**) is an alkaloid, originally isolated from *Ocotea glaziovii* (Lauraceae),[1] belonging to the fairly small proaporphine family. In the early 1970's its pharmacology was explored extensively by a pharmaceutical company (Simes S.p.A., Milano), and was registered as a tranquilizer under the trademark Suavedol®. Its psychopharmacology has been compared with that of diazepam in a double-blind clinical trial,[2] and its human pharmacokinetics have been studied.[3] It is also reported to possess anti-ulcer properties in humans.[4] Glaziovine figures in a recent list of sixty alkaloids of pharmaceutical and biological significance,[5] which

may be taken as an indication of its continued interest, but unfortunately no studies seem to have addressed its mechanisms of action.

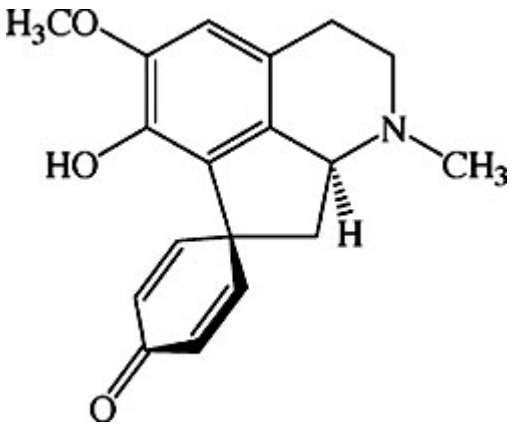

Figure 1. Structure of glaziovine (**1**).

The isolated yield of glaziovine from *O. glaziovii* leaves was 0.18-0.35 %,[1] although using two different analytical methods concentrations about ten times higher were determined.[6] However, *O. glaziovii* appears to be a rare species, and this circumstance led to an unsuccessful search for other sources of glaziovine by the pharmaceutical company that was involved in its development as a drug,[6] and in spite of the publication of several synthetic routes, some of which seemed to be fairly satisfactory,[7-12] its development was abandoned. In view of its pharmacological interest, however, a continued search for convenient, renewable natural sources of glaziovine seems warranted.

Glaziovine does not figure often in the phytochemical literature. Apart from *Ocotea glaziovii*, it has been reported as a constituent of the Lauraceous species *O. variabilis*,[13] *O. brachybotra*,[14] *Neolitsea konishii*,[15] *Litsea cubeba*,[16] *Nectandra salicifolia*,[17] and *N. pichurim*,[18] *Papaver caucasicum* (Papaveraceae),[19] *Annona purpurea* (Annonaceae),[20,21] and *Antizoma angustifolia* (Menispermaceae),[22] *O. brachybotra* leaves afforded less than 0.05 % of glaziovine.[14] In *O. variabilis*,[13] *Neolitsea konishii* and *L. cubeba*, it also appears to be very a minor constituent,[15,16] and its yield from *Nectandra salicifolia* trunk bark was in the ppm range.[17] Stems and leaves of *A. purpurea* gave less than 0.02 % of glaziovine.[20] *Papaver caucasicum* is a small plant, and unless it can be cultivated on a large scale, is uninteresting as a source of glaziovine regardless of its possible abundance in this species. The medicinal South African *Antizoma angustifolia* may be harvested in reasonable amounts, but again, glaziovine is not a major constituent and its abundance seems to be very variable.[22] On the other hand, the leaves of *N. pichurim* were reported to have afforded about 0.2 % of this alkaloid,[18] suggesting that this might be an interesting alternative source, but five years have gone by since this work was briefly described in a chemistry meeting without any subsequent publication appearing. This paper reports the presence of (6a*S*)-glaziovine as a major alkaloidal constituent leaves of *Duguetia vallicola*, a large evergreen tree from the coastal regions of Panama, Colombia and Venezuela.

EXPERIMENTAL

General Experimental Procedures. All melting points were taken on a Galen III (Cambridge Instruments) microscopic hotplate apparatus and are uncorrected. Specific optical rotation was determined using an Polartronic E (Schmidt + Haensch). ^{1}H and ^{13}C NMR spectra were acquired on either Bruker AMX 300 or Bruker AM 400 instruments at 300 and 400 MHz (^{1}H) respectively, and standard pulse sequences and parameters were used for the experiments. All chemical shift values (*d*) are given in ppm using TMS as internal standard. Silica gel 60 (Merck 0.063-0.200 mm) was used for column chromatography. Precoated silica gel 60 plates (Merck 60 F_{254} 0.2 mm) were used for TLC. TLC spots were visualized by spraying with

Dragendorff's reagent or by exposing to iodine vapor.

Plant Material. *Duguetia vallicola* (Annonaceae) was colected in Montería, Colombia at 8° 32'04.1''N, 75°40'57.9''W in December 2003 and was identified by Dr Alvaro Cogollo. A voucher specimen (JAUM 37841) is deposited in the Jardín Botánico Joaquín Antonio Uribe, Medellín, Colombia.

Extraction and Isolation. The air-dried leaves (4.50 kg) of *D. vallicola* were defatted by percolation with petroleum ether; the solid residue was then made basic with 5% aq. NH_3 solution and immediately extracted with CH_2Cl_2. The combined organic extracts were then concentrated under reduced pressure to yield 520 g of dark gummy material. The bases were redissolved CH_2Cl_2 in and extracted with 3% aq. HCl from the CH_2Cl_2 solution. The HCl solution was adjusted to pH 8-9 with concentrated aq. NH_3 and extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried over anhydrous Na_2SO_4, filtered and then concentrated to leave a brownish solid residue (30.2 g, 0.67 %). This residue was treated with methanol (500 mL) and an insoluble solid was then separated by filtration. The insoluble material was purified by "flash" CC using CH_2Cl_2/MeOH/ aq. NH_3 (80/20/1) to afford (-)-glaziovine (12 g, *ca.* 0.27 %).

(-)-Glaziovine (1): White powder, b.p. 234-237 C (reported 235-237). $[\alpha]_D$ -95 (c 0.1, $CHCl_3$). ^1H NMR data, see Table 1.

Table 1. ^1H NMR data for (-)-glaziovine (**1**) and (-)-*O*-acetylglaziovine (**3**).

Proton	(1), DMSO-d_6 (300 MHz, *J* = Hz)	(1), TFA (400 MHz, *J* = Hz)	(3), $CDCl_3$ (300 MHz, *J* = Hz)
3	6.70 s	6.90 s	6.64 s
4	3.18, 2.94	3.43 m	3.06 m
5	3.18, 2.94	3.43 m	3.48 m, 3.06 m
6a	4.29 d (10)	5.16 d (10.96)	4.40 d (12)
7	3.18 d (12.2), 2.54 t (12.2)	4.21 m, 3.09 t (12.0)	3.48 m, 2.51 q (12.0)
8	6.50 dd (2.6, 8.1)	6.78 d (8.4)	6.60 dd (2.73, 8.5)
9	5.65 dd (2.5, 8.3)	6.00 d (7.8)	5.82 dd (2.75, 8.3)
11	6.26 d (8.6)	6.47 d (8.3)	6.33 dd (1.76, 8.3)
12	7.03 d (8.3)	7.29 d (8.3)	7.16 d (8.0)
OH-1	8.32 s	8.49 s	
OCH_3-2	3.77 s	4.00 s	3.80 s
N-CH_3	2.50	2.96 s	2.55 s
OAc-1			1.61 s

(-)-Pronuciferine (2): To a suspension of (-)-glaziovine (**1**) (1 g, 3.35 mmol) in dry ether (250 mL), a recently prepared ethereal solution of diazomethane (excess) was added dropwise keeping the temperature at 0 °C. The reaction mixture was allowed to reach rt and stirred for 1 week. The unreacted diazomethane was destroyed by addition of AcOH, the solvent was removed *in vacuo* and the crude material was purified by column chromatography eluting with CH_2Cl_2/MeOH/aq. NH_3 (80/20/1). Recrystallisation from methanol gave the title compound as colorless needles (430 mg). $[a]_D$ -117 (*c* 0.3, MeOH). ^1H NMR, ^{13}C NMR, HMBC, COSY, NOESY data, see Table 2.

Table 2. NMR correlations for (-)-pronuciferine (**2**).

Atom	δ_C (ppm)	δ_H (ppm), J = Hz	HMBC	COSY	NOESY
1	150.9				
2	151.8				
3	109.2	6.54 s	125.5, 139.9, 143.6, 151.8, 150.4		2.87, 3.99, 3.78
3a	125.5				
3b	130.4				
3c	143.6				
4	24.71	2.87 m, 3.99m	44.57, 109.2, 125.5, 130.4	2.99, 3.87, 8.25	2.5, 3.33, 6.54
5	44.57	3.87 m, 3.33 m	24.71, 59.43, 125.5, 130.4	3.33, 4.24	2.87, 3.99, 2.5
6a	59.43	4.24 d (J = 10)	37.71, 42.28, 44.57, 125.5	2.99, 3.20	2.5, 3.3, 6.25, 7.01
7	37.71	2.99 dd (J = 10.5, J = 13.1), 3.20 m	125.5, 129.0, 130.4	3.20, 4.24	4.24, 6.25
7a	37.71				
8	117.3	6.57 dd (J = 2.01, J = 8.44)	113.9, 130.4, 154.1	5.80, 7.01	3.31, 5.80, 6.25, 7.01
9	113.9	5.8 dd (J = 2.5, J = 8.34)	117.3, 154.1	6.25, 6.57	3.31, 6.25, 6.57, 7.01
10	154.1				
11	128.6	6.25 dd (J = 2.27, J = 8.35)	113.9, 117.3, 128.0, 154.1, 37.71	5.80, 7.01	2.49, 4.24, 5.8, 6.57, 7.01
12	128.0	7.01 dd (J = 2.36, J = 8.01)	113.9, 37.71, 154.1	6.25, 6.57	2.5, 3.2, 4.24, 5.8, 6.57, 7.01
CH$_3$O-1	59.89	3.31	150.9		5.8, 6.57
CH$_3$O-2	55.93	3.78	151.8		6.54
NCH$_3$	43.28	2.5	59.43, 44.57		2.87, 3.99, 4.24

(-)-*O*-Acetylglaziovine (3): To a solution of (-)-glaziovine (**1**) (200 mg, 0.67 mmol) in AcOH (50 mL), acetyl chloride (0.5 mL, 6.4 mmol) was added and the mixture was refluxed with stirring for 2 h. The reaction was stopped by addition of water (2 mL), made basic with aq. NH_3 and extracted with CH_2Cl_2. The organic solution was dried over anhydrous Na_2SO_4 and the solvent was removed by distillation. The crude material was purified by column chromatography eluting with CH_2Cl_2/MeOH/aq. NH_3 (80/20/1) to obtain 200 mg of **3**, (88%). Brown powder, $[a]_D$ -177 (*c* 0.3, MeOH). ^{1}H NMR data, see Table 1.

RESULTS AND DISCUSSION

We recently published a study on the alkaloids isolated from the stem bark of *Duguetia vallicola* J. F. Macbr. (Annonaceae), which is a fairly common, large evergreen tree, growing at low altitudes in southern Panamá and in the coastal regions of Colombia and Venezuela. In that paper we reported the presence of the aporphines oliveroline and oliveridine, the oxoaporphines *O*-methylmoschatoline and duguevalline (new), and the 1-azaanthraquinone cleistopholine. Of these, cleistopholine and oliveroline showed activity against *Plasmodium falciparum* at low micromolar concentrations.[23] A reexamination of this plant material showed that the protoberberine pseudopalmatine appeared to be in fact a major alkaloidal constituent of the mixture, together with smaller amounts of its berbine precursor xylopinine and the related discretine. Furthermore, extraction of the leaves afforded 0.59 % of an alkaloid mixture from which the aporphine *N*-methyllaurotetanine was isolated as a major constituent, followed by isoboldine, isocorydine, and the berbine discretine all of which, together with the compounds isolated previously from the bark, were assayed for antioxidative activity with isoboldine and *N*-methyllaurotetanine showing the highest potencies, while pseudopalmatine and isoboldine were found to have particularly potent antiplasmodial activity.[24,25]

In the *D. vallicola* extractions mentioned above, the dry, powdered plant materials (leaves and stem bark separately) were dampened with 5 % aqueous NH_3 and dried again before extraction with CH_2Cl_2. When processing a new batch of leaves, the basified material was not dried but extracted immediately with CH_2Cl_2. Interestingly, under these conditions the composition of the crude alkaloid fraction was quite different and upon treating it with methanol an insoluble material separated which proved to be the major constituent, and after purification afforded (6a*S*)(-)-glaziovine amounting to *ca.* 40 % of the alkaloidal fraction of the leaves. This corresponds to an isolated yield of glaziovine of about 0.27 %, well within the range reported for *O. glaziovii*.

Glaziovine was identified on the basis of its ^{1}H NMR spectra in DMSO-d_6 and in trifluoroacetic acid (Table 1). Its low solubility in these and other common NMR solvents, as well as its abundance, led to the preparation of its *O*-acetyl and *O*-methyl (pronuciferine) derivatives that

were subjected to additional NMR studies. Two-dimensional ^{1}H-^{1}H COSY, NOESY, and HSQC/HMBC experiments performed on pronuciferine allowed, for the first time, the unambiguous assignment of all the ^{1}H and ^{13}C NMR signals of a propaporphine. Figure 2 summarizes the ^{1}H and ^{13}C NMR chemical shifts of pronuciferine. Table 2 repeats these values, showing the results of the HMBC, COSY and NOESY experiments.

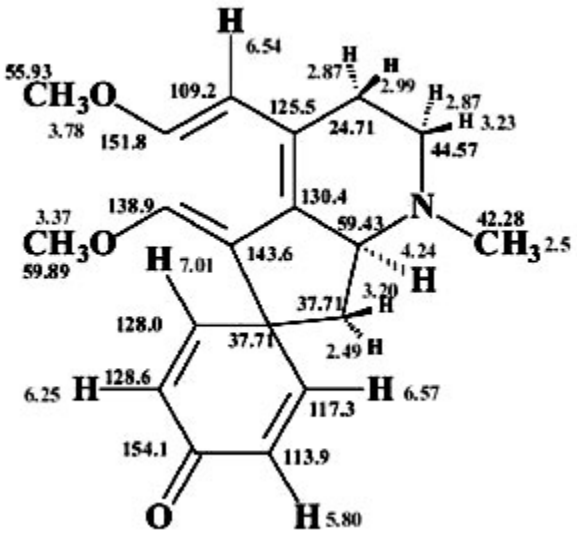

Figure 2. Summary of ^{1}H and ^{13}C NMR chemical shifts of (-) pronuciferine (**2**).

The material isolated originally from *O. glaziovii* was reported to have an $[\alpha]_D$ value of +7,[1] and the alkaloid isolated from *O. variabilis* and *O. brachybotra* was claimed to be racemic.[13,14] The specific rotation of the glaziovine isolated from *Annona purpurea* does not seem to have been determined,[20,21] and the same appears to be true for the alkaloid from *Neolitsea konishii*,[15] *Litsea cubeba*,[16] *Nectandra pichurim*,[18] *Papaver caucasicum* (Papaveraceae),[19] and *Antizoma angustifolia.*[22] Our material was levorotatory with $[\alpha]_D$ = -95 in methanol and gave a similarly levorotatory *O*-acetyl derivative and was *O*-methylated to afford (-)-pronuciferine, clearly establishing its (at least predominantly) (6a*S*) stereochemistry, as is the case for the alkaloid isolated from *N. salicifolia*.[17]

The presence of either or both enantiomers of glaziovine in different plant species is intriguing from a biogenetic point of view. The (*R*) and (*S*) isomers should arise by phenolic oxidative coupling of (6a*R*)- and (6a*S*)-*N*-methylcoclaurine, respectively, and the coexistence of both coclaurine enantiomers in a particular plant is well documented.[26,27] Nevertheless, only (*S*)-norcoclaurine synthase has been characterized, possibly suggesting that (*R*)-coclaurine arises from the facile non-enzymatic Pictet-Spengler condensation of dopamine and (4-hydroxyphenyl)acetaldehyde and that the subsequent *N*- and *O*-methylations are not stereospecific.[28-30] By extension of this reasoning, the coupling reaction leading to (*R*)- or (*S*)-glaziovine should also be non-stereospecific. However, all known aporphine alkaloids believed to be derived from proaporphines have the (6a*R*) stereochemistry, indicating that the dienone-phenol and dienol-benzene rearrangements only occur naturally with substrates of this absolute configuration.

Our results have now revealed that *D. vallicola* leaves are an abundant, renewable source of the potentially useful anxiolytic (6a*S*)(-)-glaziovine, comparable in yield to the leaves of the rare *Ocotea glaziovii*, but with strong predominance of one of the enantiomers.

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