(*R*)-and (*S*)-coclaurine from the bark of *Peumus boldus*

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SUMMARY. *The stem bark of* P. boldus, *in addition to the previously reported benzylisoquinoline alkaloids* sensu lato, *accumulates small amounts of their common precursor* (R)- *and (to a lesser extent)* (S)-*coclaurine. The enantiomeric composition of the crude coclaurine mixture was estimated to be approximately (6:1) on the basis of the optical rotation of the crystallised alkaloid.*

Key words: *Peumus boldus; (R)-* and *(S)*-coclaurine.

The fundamental role of (*R*) and (*S*)-coclaurine in the biosynthesis of benzylisoquinoline alkaloids in the broadest sense (*i.e.*, including dimers, aporphinoids, berbinoids, morphinoids, etc.) has only been recognized rather recently.[1,2] Among the biological materials used in these studies, cell cultures of *Peumus boldus* Mol. (Monimiaceae) provided additional evidence for the incorporation of (*S*)-norcoclaurine, *via* (*S*)-coclaurine, into the central benzylisoquinoline intermediate (*S*)-reticuline,[2,3] in this case in a plant species which utilizes the latter alkaloid as a precursor of its abundant aporphinoids.[4] In spite of the proven intermediacy of coclaurine in *P. boldus* cell cultures, this plant material has only been shown to accumulate the noraporphines laurolitsine (= norboldine) and laurotetanine in isolable amounts.[3] In this regard, it is interesting to note that noraporphines appear to be *N*-demethylation products of aporphines, and not their precursors.[3]

The medicinal boldo (*P. boldus*) leaves have been extensively analyzed for alkaloids and are a well-known source of boldine,[5] isocorydine, norisocorydine, *N*-methyllaurotetanine,[6] reticuline, isoboldine,[7] laurotetanine, laurolitsine,[8] and isocorydine *N*-oxide.[9] More recently, the stem bark has been shown to contain boldine (by far the major alkaloidal constituent in this tissue), isocorydine, *N*-methyllaurotetanine, norisocorydine and the morphinandienone sinoacutine, all presumably (*S*)-coclaurine metabolites, and the proaporphine (-)-pronuciferine,[10] derived from (*R*)-coclaurine, plus 6a,7-dehydroboldine.[11] The wood contains laurolitsine and at least eight other unidentified bases.[12] In this context, it seems somewhat surprising that coclaurine should never have been reported as a boldo constituent.

The specific rotations for coclaurine samples isolated from different plant sources show considerable variations. It has been suggested that the original material from *Cocculus laurifolius* (Menispermaceae) was dextrorotatory,[13] but other samples from *Cocculus* and *Machilus* are thought to have been racemic.[14] Materials crystallized from *Alseodaphne archboldiana* and *Xylopia papuana* showed $[\alpha]_D$ values (in ethanol) of + 22° and + 47°, respectively,[14] and the latter was shown to have the (*S*) configuration on the basis of a single crystal X-ray crystallographic study.[15] A sample crystallized from *Unonopsis stipitata* showed $[\alpha]_D$ + 30° (in methanol),[16] and should therefore be mainly (*S*). Considering that both (*R*)- and (*S*)-coclaurine ought to be present as precursors of more elaborate

structures with both absolute configurations, this variability is not surprising. As usual in tradition natural products chemistry, these products have been crystallized several times whenever possible, thus disturbing their initial enantiomeric compositions. Nevertheless, the sizes of the metabolic pools of both stereoisomers and the quantitative aspects of their interconversion can only be estimated with some accuracy if the concentration of each is measured in the isolated mixture before any abiotic chiral selection can take place.

EXPERIMENTAL

Flash chromatography of boldine mother liquors. On silica gel for TLC, eluting with $CHCl_3$ containing increasing concentrations of MeOH. Glaucine elutes at 4% MeOH, isocorydine at 6%, boldine at 8%, and coclaurine (followed closely by laurolitsine) at 20-30%.

Coclaurine [mainly (R)]. The material eluted from the column and aligning on TLC with (±)-coclaurine (synthesised according to Teitel and Brossi[17]) was converted into its hydrochloride and recrystallized in water: colorless prisms, mp (H_2O) 250-255°C. The base recovered from the partially purified salt was crystallized twice in EtOH. Off-white microcystals, mp (EtOH) 208-210°C (lit.[14] 217-218°C); $[\alpha]_D$ −34° (c 0.4, EtOH) (lit.[14,16] +22°, +47°, +30°); ^{1}H-NMR δ (60 MHz, CD_3OD-$CDCl_3$ 2:1) 3 (6H, *m*, H-3/4/9), 3.86 (3H, *s*, OCH_3), 4.1 (1H, *m*, H-1), 6.70 (1H, *s*, H-5), 6.80 (1H, *s*, H-8), 6.82 (2H, *d*, *J* 8.5 Hz, H-2'/6'), 7.16 (2H, *d*, *J* 8.5 Hz, H-3'/5'); ^{1}H-NMR δ (300 MHz, CD_3OD) 2.8 (4H, *m*, H-3/4), 3.13 (1H, *dd*, *J* 16.0 Hz, *J'* 4.1 Hz, H-9α), 3.18 (1H, *dd*, *J* 16.0 Hz, *J'* 5.7 Hz, H-9β), 3.81 (3H, *s*, OCH_3), 4.04 (1H, *dd*, *J* 9.3 Hz, *J'* 4.1 Hz, H-1), 6.64 (1H, *s*, H-8), 6.68 (1H, *s*, H-5), 6.75 (2H, *d*, *J* 8.4 Hz, H-10/14), 7.07 (2H, *d*, *J* 8.4 Hz, H-11/13); ^{13}C-NMR δ (75 MHz, CD_3OD) 29.73 (C-4), 41.72 (C-3 or C-9), 42.42 (C-9 or C-3), 56.75 (O-CH_3 or C-1), 58.24 (C-1 or O-CH_3), 113.39 (C-8), 114.45 (C-5), 116.82 (C-11/C-13), 127.09 (C-8a), 130.61 (C-4a), 131.41 (C-9a), 131.65 (C-10/C-14), 146.06 (C-7), 148.21 (C-6), 157.55 (C-12).

(±)-Coclaurine. ^{1}H-NMR δ (300 MHz, DMSO-d_6) 2.6 (2H, *m*, H-9), 2.68 (1H, *dd*, *J* 13.4 Hz, *J'* 3.3 Hz, H-3 or H-4), 2.78 (1H, *ddd*, *J* 11.7 Hz, *J'* = *J''* 5.7 Hz, H-3 or H-4), 2.99 (1H, *dd*, *J* 13.6 Hz, *J'* 3.3 Hz, H-4 or H-3), 3.10 (1H, *ddd*, *J* 11.7 Hz, *J'* = *J''* 5.7 Hz, H-4 or H-3), 3.77 (3H, *s*, OCH_3), 3.92 (1H, *dd*, *J* 9.3 Hz, *J'* 3.1 Hz, H-1), 6.63 (1H, *s*, H-8), 6.71 (1H, *s*, H-5), 6.75 (2H, *d*, *J* 8.2 Hz, H-10/14), 7.10 (2H, *d*, *J* 8.2 Hz, H-11/13); ^{13}C-NMR δ (75 MHz, DMSO-d_6) 28.99 (C-4), 39.98 (C-3, overlapping solvent signal), 41.36 (C-9), 55.75 (O-CH_3), 56.54 (C-1), 112.45 (C-8), 113.59 (C-5), 115.29 (C-11/C-13), 125.58 (C-8a), 129.65 (C-4a), 130.47 (C-10/C-14), 131.12 (C-9a), 144.49 (C-7), 146.21 (C-6), 155.89 (C-12).

Coclaurine [mainly (R)] (R)-α-methoxy-α-(trifluoromethyl)phenylacetyl derivative. Crude coclaurine from the column was treated with an excess of (*R*) (+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride in DMSO-d_6 and allowed to react overnight: ^{13}C-NMR δ (75 MHz, DMSO-d_6) 53.41 ppm ([*R*]-C-1).

(±)-Coclaurine (R)-α-methoxy-α-(trifluoromethyl) phenylacetyl derivative. (±)-Coclaurine was treated in the same way as the natural product with (*R*) (+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride: ^{13}C-NMR δ (75 MHz, DMSO-d_6) 53.48 ([*R*]-C-1), 55.59 ppm ([*S*]-C-1).

RESULTS AND DISCUSSION

While working on the fractionation of the chloroform mother liquors of boldine from boldo bark, it seemed worthwhile to look for coclaurine in the more polar alkaloid fraction which tends to be neglected owing to its less facile work-up. A TLC spot running barely above the start line with solvent systems ap-

propriate for the separation of boldine and other major constituents appeared to be a good candidate for investigation. Using the more polar eluent chloroform-methanol 6:4, this spot aligned nicely with synthetic (±)-coclaurine prepared according to a now classical procedure.[17] Isolation of this minor boldo bark alkaloid and comparison of its physical and spectral characteristics with those published for (mainly *S*)(+)-coclaurine [14,16] and the racemate [17] established its identity as an enantiomeric mixture which in the twice crystallized material - $[\alpha]_D$-34° (c 0.4, EtOH) - contains about 86% (*R*)(-)- and about 14% (*S*)(+)-coclaurine assuming that $[\alpha]_D$ + 47° corresponds to the pure (*S*) isomer.

As the recrystallized coclaurine could be expected to be enriched in either the racemate or the dominant isomer, an attempt was made to determine the enantiomeric composition of the alkaloid as obtained directly from the chromatographic column. This material was derivatised with one equivalent of (*R*)(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl (MTPA) chloride [18] and analysed by high-field ^{13}C-NMR. The expected splitting of the C(1) signal at 53.41 ppm (in DMSO-d_6) could not be observed, suggesting that the material from the *P. boldus* bark contains too little of the minor enantiomer for it to be quantifiable in a fairly clean natural-abundance ^{13}C-NMR spectrum at 75 MHz. After derivatising (±)-coclaurine in a similar fashion, the corresponding signals were visible at 53.48 and 55.59 ppm. It should be remembered that in C-1-^{13}C-enriched coclaurine [2] a diastereomeric shift of about 2 ppm may be seen for the resonances of the chiral carbon atoms.

It would seem reasonable to expect (*S*)- and (*R*)-coclaurine to be metabolised very efficiently to reticuline, tetraoxygenated aporphinoids and probably sinoacutine (although the configuration of this alkaloid from *P. boldus* was not determinated) [12] on one hand, and to pronuciferine on the other. The metabolic pools of both coclaurine isomers could be expected to be very small, and it is therefore of interest that both should be accumulated in easily isolable amounts in bark tissue, a fact suggesting that they may contribute together with other secondary metabolites to the plant's defense against predators and parasites. *P. boldus* appears to be unusual, however, in that it stores larger amounts of the (*R*) isomer. This could be a consequence of the fact that *P. boldus* does not seem to use (*R*)-coclaurine to any great extent, considering that boldine - the major bark alkaloid - and most of the other bases isolated from both bark and leaves, are (*S*)-coclaurine metabolites. Such a situation contrasts with what occurs, for example, in *Unonopsis stipitata*, where the (*R*)-coclaurine-derived (-)-curine, argentinine and stipitatine abound. Although similar data are lacking regarding *Xylopia papuana*, it would seem reasonable to expect the presence of typical annonaceous (*R*)-coclaurine metabolites such as anonaine and liriodenine in considerable amounts in association with the predominantly (*S*)-coclaurine.

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