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SOME DIMERIC BENZYLISOQUINOLINE ALKALOIDS WITH AN UNUSUAL OXYGENATION PATTERN*

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ABSTRACT.—*Berberis buxifolia* Lam. (Berberidaceae) has yielded the new bisbenzylisoquinolines chillanamine (**3**) and (−)-osornine (**4**), as well as the new secobisbenzylisoquinolines (−)-curacautine (**10**) and (−)-talcamine (**11**). Cleavage of (−)-*O*-methylosornine (**5**) with sodium in liquid ammonia led to tetrahydrobenzylisoquinolines (+)-**6** and (−)-**7**. A close relative of (−)-osornine is the known calafatine (**1**), now reisolated from *B. buxifolia*. This alkaloid is levo- rather than dextro-rotatory, and its sodium-in-liquid-ammonia cleavage provided (+)-**8** and (−)-**9**. Potassium permanganate oxidation of **1** gave rise to **10**. Biogenetic considerations imply that two *N*-methylcoclaurines first combine in tail-to-tail fashion. The resulting dimer is then oxidized enzymically *ortho* to the diaryl ether bridge to supply the C-10 oxygenated function. *O*-Methylation at an appropriate stage furnishes **3**. Alkaloid **3** is the likely precursor of **4** and **1**. The latter suffers oxidative cleavage to (−)-curacautine, which can then be transformed into (−)-talcamine.

A number of bisbenzylisoquinolines have come to light in recent years which incorporate an extra oxygen in ring C (1). These unusual bases may be considered variants of either calafatine (**1**) (2), whose absolute configuration was unknown at the start of the present study, or (+)-thalibrunine (**2**) (3). Calafatine and its congeners are found in *Berberis* species (Berberidaceae), whereas thalibrunine-type bases have been detected only in *Thalictrum* species (Ranunculaceae).

We have investigated the alkaloidal content of *Berberis buxifolia* Lam., collected in Chilean Patagonia. The first new alkaloid we report is the triphenolic chillanamine (**3**), $C_{37}H_{42}O_7N_2$. The mass spectrum of this material exhibited a simple pattern including a small molecular ion, *m/z* 626, and a dominating base peak, *m/z* 192, due to portion *a* of the dimer. The nmr spectrum has been summarized around expression **3** and supplied nearly definitive information regarding the structure of the alkaloid. A key feature is the two downfield doublets centered at δ 6.58 and 6.69 (J_o=8.5 Hz) representing the two aromatic hydrogens of ring C. Additionally, an nmr noe study showed that irradiation of the C-12 methoxyl singlet at δ 3.91 resulted in 6.8% enhancement of the δ 6.69 doublet (H-13), so that the hydrogens involved must be proximate.

Chillanamine (**3**) should be juxtaposed with the second new alkaloid, (−)-osornine (**4**), which is monophenolic and analyses for $C_{38}H_{42}O_7N_2$. The mass spectrum exhibits molecular ion peak *m/z* 638. The base peak *m/z* 381 represents the *a* portion of the molecule minus a hydrogen. The nmr spectrum of osornine (**4**) is summarized in table 1. It will be noted that there are two downfield doublets at δ 6.74 and 7.16 (J_o=8.6 Hz) representing H-13 and -14, reminiscent of a similar pattern in the spectrum of **3**. Diazomethane *O*-methylation of **4** led to (−)-*O*-methylosornine (**5**), $C_{39}H_{44}O_7N_2$, whose nmr spectrum includes an extra methoxyl singlet at δ 2.97. Because this spectrum is better defined than that of osornine (**4**), it has been summarized around expression **5**. The results of a detailed nmr noe study of *O*-methylosornine (**5**) are given in the table. Of particular importance is the 1% noe of H-13′ (δ 7.00) observed upon irradiation of the C-10 methoxyl (δ 3.81), and the 2.3% noe of H-8 (δ 4.79) resulting from irradiation of the C-7′ methoxyl (δ 2.97). Cleavage of *O*-methylosornine (**5**) with sodium in liquid ammonia by an improved procedure (see Experimental) led to the tetrahyd-

*This paper is dedicated to Dr. Arnold Brossi on the occasion of his 60th birthday.

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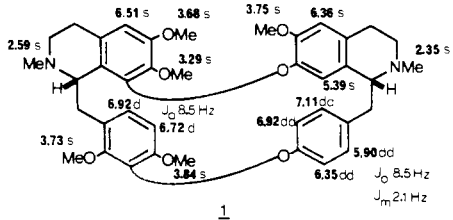

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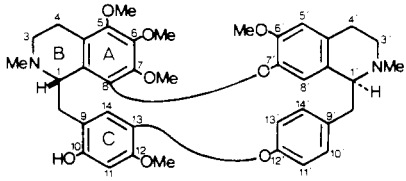

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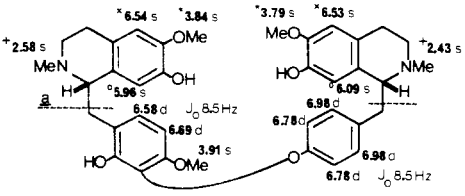

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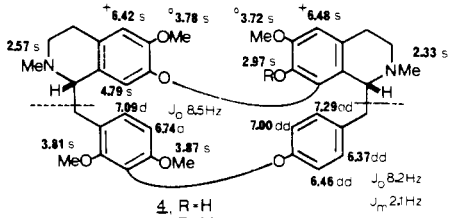

4, R = H
5, R = Me
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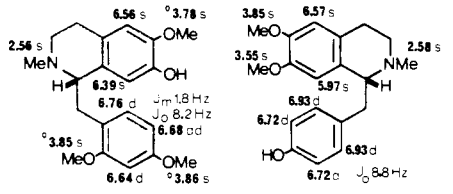

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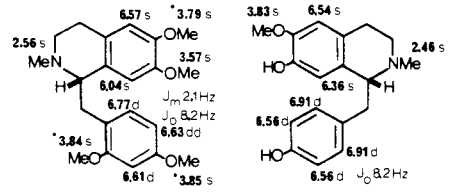

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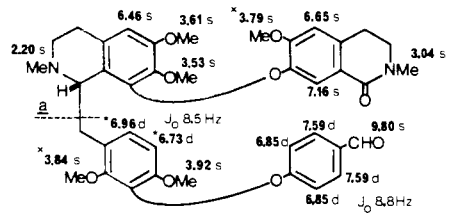

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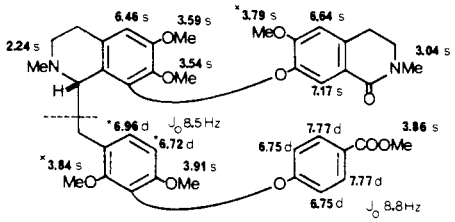

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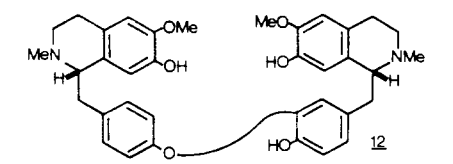

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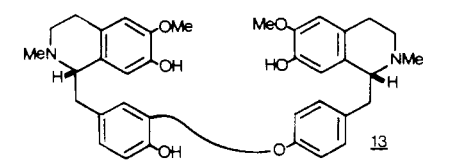

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robenzylisoquinoline (+)-**6** and (−)-armepavine (**7**), indicating that the stereochemistry of **4** and **5** is as shown.

A close relative of (−)-osornine (**4**) is calafatine (**1**) (2), which we have now reisolated from *B. buxifolia* and which is structurally isomeric with (−)-*O*-methylosornine (**5**). The original measurement of the specific rotation of this alkaloid was done on an old and unreliable polarimeter that gave a positive value. We have ascertained that calafatine has $[\alpha]^{25}D - 154°$ (*c* 0.28, $CHCl_3$). Its absolute configuration is identical to that of (−)-osornine, because in our hands, sodium-in-liquid-ammonia reduction of calafatine led to (+)-**8** as well as to (−)-*N*-methylcoclaurine (**9**).

(−)-Curacautine (**10**), $C_{39}H_{42}O_9N_2$, is a new secobisbenzylisoquinoline present in *B. buxifolia*, ν max ($CHCl_3$) 1610, 1640 and 1690 cm^{-1}. The mass spectrum shows a

TABLE 1. Physical and spectral data.

(−)-Calafatine (**1**): CD Δε(nm) 0(300), +9(247), 0(242), −18(234), 0(225), +10(219), 0(212); $[\alpha]^{25}D$ −154° (c 0.28, $CHCl_3$).

Chillanamine (**3**): λ max 209, 226 sh, 283 nm (log ε 4.81, 4.54, 4.02); ms *m/z* 626 (M^+) (0.1), 625 (0.1), 192 (100), 177 (18); CD Δε(nm) 0(300), +0.5(271), 0(265), −6(230), 0(220), +8.3(212).

(−)-Osornine (**4**): mp 244-245° (MeOH); λ max 207, 230 sh, 282 nm (log ε 4.80, 4.53, 3.75); ms *m/z* 638 (M^+) (47), 381 (100), 191 (79), 174 (22); CD Δε(nm) 0(300), +0.6(282), 0(276), −2(270), 0(250), +41(232), 0(219), −60(209); $[\alpha]^{25}D$ −151° (c 0.36, $CHCl_3$); nmr δ 2.31, 2.46 (2×NCH_3), 3.71, 3.82, 3.83, 3.88 (4×OCH_3), 4.60 (1H, s), 6.46 (1H, s), 6.48 (1H, s), 6.65 (2H, br s), 6.74 (1H, d, J_o=8.8 Hz), 6.90 (1H, dd, J_o=8.2 Hz, J_m=1.8 Hz), 7.16 (1H, d, J_o=8.5 Hz), 7.29 (1H, dd, J_o=8.2 Hz, J_m=2.1 Hz).

(−)-*O*-Methylosornine (**5**): ms *m/z* 652 (M^+) (42)], 395 (68), 198 (100), 174 (36); nmr nOe N′Me (δ 2.33)↛H-1′ (δ 4.02) (8%); NMe (δ 2.57)↛H-1 (δ 4.48) (7.5%); 7′-OMe (δ 2.97)↛H-8 (δ 4.79) (2.3%); 10-OMe (δ 3.81)↛H-13′ (δ 7.00) (1%); 12-OMe (δ 3.87)↛H-13 (δ 6.74) (16.6%); H-1′ (δ 4.02)↛H-14′ (δ 7.29) (2.3%); H-1 (δ 4.48)↛NMe (δ 2.57) (1.5%), H-8 (δ 4.79) (4%), H-13′ (δ 7.00) (5%); H-8 (δ 4.79)↛H-1 (δ 4.48) (4.6%), 7′-OMe (δ 2.97) (0.95%); H-13 (δ 6.74)↛12-OMe (δ 3.87) (1.5%), H-14 (δ 7.09) (3.5%); H-13′ (δ 7.00)↛H-1 (δ 4.48) (2.8%), H-14′ (δ 7.29) (7.5%).

Tetrahydrobenzylisoquinoline (+)-**6**: λ-max 205, 231 sh, 283 nm (log ε 4.52, 4.04, 4.47); ms *m/z* 343 (M^+) (0.2), 342 (0.5), 192 (100), 151 (5); CD Δε (nm) 0(300), +0.7(285), 0(270), +1.7(237), +0.7(222), +6(210); $[\alpha]^{25}D$ +22° (c 0.25, MeOH).

(−)-Armepavine (**7**): CD Δε (nm) 0(300), −0.94(287), 0(270), −5.3(232), −3.2(221), −7(208); $[\alpha]^{25}D$ −57° (c 0.33, MeOH).

Tetrahydrobenzylisoquinoline (+)-**8**: λ max 205, 232 sh, 282 nm (log ε 4.58, 4.05, 3.58); ms *m/z* 357 (M^+) (0.1), 206 (100); CD Δε (nm) 0(300), +1.4(285), 0(270), +3.6(236), +1.6(225), +9(211); $[\alpha]^{25}D$ +47° (c 0.27, MeOH).

(−)-*N*-Methylcoclaurine (**9**): CD Δε (nm) 0(300), −1.5(285), 0(275), −7.2(229), −3.8(216), −10.7(207); $[\alpha]^{25}D$ −72° (c 0.29, MeOH).

(−)-Curacautine (**10**): λ max 207, 223 sh, 271, 282 nm (log ε 4.85, 4.74, 4.35, 4.29); ms *m/z* 682 (M^+) (0.1), 681 (0.1), 411 (100), 365 (11), 271 (0.3), 206 (2.2), 204 (6); CD Δε (nm) 0(320), −1.5(300), 0(290), +1.7(285), +3(263), 0(249), −16(230), 0(218), +4.3(214); $[\alpha]^{25}D$ −5° (c 0.18, MeOH).

(−)-Talcamine (**11**): λ max 208, 225 sh, 260, 272 sh, 305 nm (log ε 4.93, 4.79, 4.41, 4.27, 3.67); ms *m/z* 712 (M^+) (0.1), 681 (0.4), 653 (0.1), 411 (100), 365 (9), 301 (0.4), 206 (2); CD Δε (nm) 0(320), −3(295), 0(266), +4(252), 0(245), −23(230), 0(221), +14(214); $[\alpha]^{25}D$ −2° (c 0.29, MeOH).

small molecular ion, *m/z* 682, and a prominent base peak, *m/z* 411, due to the *a* portion of the molecule. The cd curve with a maximum at 214 nm is reminiscent of a tetrahydrobenzylisoquinoline of the *S* configuration (4). Finally, potassium permanganate in acetone (5) oxidation of calafatine (**1**) provided (−)-curacautine (**10**) identical with the natural product.

The last alkaloid we describe is (−)-talcamine (**11**), $C_{40}H_{44}O_{10}N_2$, ν max ($CHCl_3$) 1605, 1640 and 1710 cm^{-1}, which is the methyl ester of curacautine (**10**). The mass spectrum shows a small molecular ion, *m/z* 712, and a prominent base peak, *m/z* 411, identical to that for **10**, and due to portion *a*. The cd curves of **10** and **11** (table 1) closely resemble each other, reflecting the identical configuration.

The chirality of chillanamine (**3**) was not discussed above because lack of sufficient material precluded a sodium-in-liquid-ammonia cleavage. We were able, however, to record the cd spectrum. Perusal of the structures for calafatine (**1**), chillanamine (**3**), osornine (**4**), curacautine (**10**), and talcamine (**11**), strongly suggests that all of these alkaloids are biogenetically interrelated. It follows that chillanamine (**3**) must incorporate the same absolute configuration as its companions, so that H-1 and H-1′ are located above the mean plane of the molecule as drawn. (It is also worth pointing out that all bisbenzylisoquinolines isolated from the Berberidaceae, which include the alkaloids berbamine, berbamunine, oxyacanthine, himanthine, isotetrandrine, belarine, espinidine, espinine, lauberine, *O*-methylisothalicberine, obaberine, 2′-*N*-methylber-

bamine, oblongamine, obamegine, aromoline, baluchistine, calafatine, 7-*O*-demethylisothalicberine, isothalicberine, and thalrugosine incorporate the absolute configuration 1*R*,1′*S* or 1*S*,1′*R*, *i.e.*, the hydrogens at the asymmetric carbons are *syn* to each other, being either both above or below the plane of the molecule).

The characterization of the above new natural products provides us with an interesting insight into some of the biogenetic sequences that occur in the plant. Chillanamine (**3**) must undergo intramolecular oxidative coupling to supply, after *O*-methylation, either osornine (**4**) or calafatine (**1**). The latter can suffer oxidative cleavage to curacautine (**10**), whose further oxidation and esterification produces talcamine (**11**).

The key problem remaining at this stage concerns the origin of the extra C-10 oxygen function present in all of these species. This question can be answered with some degree of certainty because monomers of types **6** and **8** never have been isolated from plant sources. Species **6** or **8** would have to originate from an *N*-methylcoclaurine unit that undergoes *meta* oxidation in the bottom ring, and such oxidation is unknown in nature. The conclusion is, therefore, that two *N*-methylcoclaurines combine together in tail-to-tail fashion, and the resulting dimer is then oxidized enzymatically *ortho* to the diaryl ether to supply the C-10 oxygenated function. *O*-Methylation at some appropriate stage would then furnish chillanamine (**3**). In the case of thalibrunine (**2**) and its analogs, enzymic oxidation must occur *para*, rather than *ortho*, to the diaryl ether oxygen.

A further point of interest revolves around the direction of the initial diaryl ether bridge formed by the condensation of the two *N*-methylcoclaurines. Either (+)-berbamunine (**12**) or its enantiomer (−)-magnoline (**13**) may result from this dimerization, and both modes may occur within *Berberis* species. However, only berbamunine (**12**) can undergo further phenolic oxidation to proaporphine-benzylisoquinoline dimers of the pakistanamine series, which, in turn, rearrange to aporphine-benzylisoquinolines (6). Magnoline (**13**), on the other hand, pursues a different course, with one of the avenues followed involving oxidation *ortho* to the diaryl ether bridge accompanied by *O*-methylation to supply chillanamine (**3**). The remaining biogenetic sequence from chillanamine has already been delineated above.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The uv and cd spectra were recorded in methanolic solutions; nmr spectra are in $CDCl_3$ at 360 MHz (FT) (in the diagrams above, nmr chemical shifts with identical superscripts are interchangeable); tlc Rf values for the new alkaloids on Merck Silica Gel F-254 glass plates using the system $CHCl_3$-MeOH-NH_4OH (95:5:0.5 v/v) are calafatine (**1**) 0.34, osornine (**4**) 0.19, curacautine (**10**) 0.54, talcamine (**11**) 0.55, and chillanamine (**3**) 0.06. All compounds are amorphous unless specified otherwise.

FRACTIONATION OF *B. BUXIFOLIA* ALKALOIDAL EXTRACTS.—The crude tertiary alkaloids (18 g) from extraction of 8 kg of dried and powdered plant material was dissolved in a minimum amount of chloroform and loaded on a large column packed with 1.1 kg Silica Gel 60 (Merck 70-230 mesh) in chloroform. Initial elution was with chloroform and then with increasing proportions of methanol in chloroform. Fractions of about 1 liter were collected and their contents followed by tlc. The spots were visualized by either long or short wave-length uv light, and also by spraying with the chloroplatinate or Dragendorff spray reagents.

CURACAUTINE (**10**) AND TALCAMINE (**11**).—Fraction 60, eluted with 2% methanol in chloroform, contained relatively large amounts of a yellow solid. Following filtration, the filtrate (880 mg after evaporation of solvent) was chromatographed on a small Silica Gel 60H (Merck) column, using chloroform-methanol-ammonium hydroxide (95:5:0.5) as the eluting solvent. Fractions measuring 5-30 ml were collected. Fractions 4-5 were combined (64 mg) and subjected to preparative tlc first using chloroform-methanol-ammonium hydroxide (95:5:0.5), and then benzene-chloroform-diethylamine (5:4:1). Further tlc employing benzene-methanol (90:10) and benzene-diethylamine (90:10) provided curacautine (4.3 mg) and talcamine (4.7 mg).

CALAFATINE (**1**).—Fractions 71-84, eluted with 5% methanol in chloroform, were combined (2.5 g after solvent evaporation) and chromatographed on a Silica Gel 60H column with chloroform-methanol-ammonium hydroxide (95:5:0.5) as eluent. Portions measuring 15-25 ml were collected. Fractions 18-32 were combined to supply 1.2 g of calafatine.

OSORNINE (**4**).—Fractions 56-63 of the small Silica Gel 60H column used in the isolation of calafatine were combined (210 mg after solvent evaporation) and purified by tlc using chloroform-methanol-ammonium hydroxide (90:10:1). Osornine (150 mg) was thus obtained.

CHILLANAMINE (**3**).—Fractions 131-134, eluted with 10% methanol in chloroform, were combined (546 mg after solvent evaporation), and a portion (360 mg) was chromatographed on a thin layer plate using chloroform-methanol-ammonium hydroxide (90:10:1). Further purification of the major alkaloidal band by tlc, employing acetonitrile-benzene-ethyl acetate-methanol-ammonium hydroxide (40:30:20:5:5), afforded 3 mg of chillanamine.

SEMI-SYNTHESIS OF CURACAUTINE (**10**).—Calafatine (**1**) (30 mg, 0.046 mmol) was dissolved in 30 ml acetone. A solution of potassium permanganate (30 mg, 0.19 mmol) in acetone (20 ml) was added dropwise over 0.5 h with stirring. After 5 h, the reaction mixture was filtered, the solvent evaporated, and the residue purified by tlc to give 1 mg of amorphous secocalafatine aldehydolactam (3%), identical with natural curacautine.

SODIUM-IN-LIQUID-AMMONIA REDUCTION OF CALAFATINE (**1**).—The following procedure represents an improvement over the common procedure for the cleavage of bisbenzylisoquinolines using sodium in liquid ammonia. In a 100-ml three-neck flask, calafatine (100 mg) was dissolved in 10 ml THF, and 30 ml of NH_3 was added at $-78°$ (dry ice in acetone). The reaction vessel was equipped with a Dewar-type condenser, and the system was kept under nitrogen. A *minimum* amount of sodium was added with stirring, so as to produce a stable blue color lasting for at least 0.5 h. Upon addition of all the sodium (75 mg), the characteristic blue color had been maintained for a total of 2 h, by which time tlc indicated that all of the dimer had been consumed. The reaction mixture was then allowed to warm up to room temperature, and excess methanol was added to destroy any residual sodium. Following removal of the solvent, the residue was treated with water and acidified with 10% hydrochloric acid. The mixture was then basified with ammonium hydroxide, and extracted with chloroform. The organic phase was dried, the solvent evaporated, and the residue submitted to tlc using chloroform-methanol-ammonium hydroxide (90:10:1). In this manner, **8** (5.5 mg) and **9** (31 mg) were obtained.

DIAZOMETHANE *O*-METHYLATION OF OSORNINE (**4**).—A sample of osnornine (25 mg) was dissolved in 2 ml methanol, and excess diazomethane in methylene chloride was added. The contents were allowed to stand in the refrigerator for 24 h. Work-up provided **5** (21 mg, 82%).

SODIUM-IN-LIQUID-AMMONIA REDUCTION OF *O*-METHYLOSORNINE (**5**).—To a chilled ($-78°$) 25-ml three-neck flask, equipped with a Dewar condenser and a nitrogen gas inlet, was added 8.5 mg of **5** dissolved in 10 ml THF. Approximately 10 ml liquid ammonia was added, followed by the slow addition of sodium (30 mg) in sufficient amounts to produce a stable blue color for a total of 0.5 h, by which time tlc indicated that all the dimer had been consumed. Work-up, including tlc, gave rise to **6** (2.8 mg), and **7** (3 mg).

ACKNOWLEDGMENTS

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