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AMIDES OF *OTTONIA CORCOVADENSIS*

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Key Word Index—*Ottonia corcovadensis*; Piperaceae; amides; piperovatine; piperlonguminine; isopiperlonguminine; corcovadine; isocorcovadine; ^{1}H NMR and mass spectrometry.

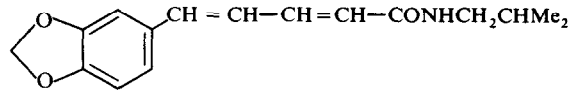
Abstract—Five amides were isolated from the roots of *Ottonia corcovadensis*. Two are the known piperlonguminine and piperovatine. The other three are new and their structures are: (2*Z*,4*Z*)-*N*-isobutyl-5-(3,4-methylenedioxyphenyl)-penta-2,4-dienoic amide for isopiperlonguminine; (2*E*,4*E*)-*N*-(2-methyl-2-acetoxypropyl)-5-(3,4-methylenedioxyphenyl)-penta-2,4-dienoic amide for corcovadine; and (2*Z*,4*Z*)-*N*-(2-methyl-2-acetoxypropyl)-5-(3,4-methylenedioxyphenyl)-penta-2,4-dienoic amide for isocorcovadine.

INTRODUCTION

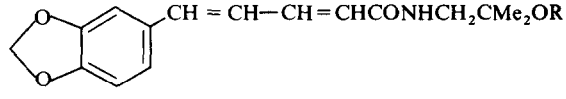
Ottonia corcovadensis Miq., a herbaceous shrub of the Piperaceae, is known in Brazilian folk medicine under the name of 'false jaborandi'.* Its roots and, to a lesser extent, the stems are chewed to alleviate toothache, due to their anesthetic action on the mucous membranes of the mouth. Extraction of the roots with petrol followed by extensive column chromatography yielded five amides. Piperlonguminine (**1**) and piperovatine (**5**) were identified by direct comparison with authentic samples. Piperovatine is the sole substance responsible for the anesthetic activity and was obtained pure by crystallization in a system of solvents at low temperature. The remaining three amides are new, having been named isopiperlonguminine (**2**), corcovadine (**3**) and isocorcovadine (**4**). Piperlonguminine–isopiperlonguminine and corcovadine–isocorcovadine are shown to be pairs of *cis–trans* isomers.

RESULTS AND DISCUSSION

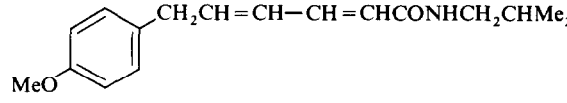
The work was performed with two batches of roots of *O. corcovadensis* collected at the same site with a 1 year interval. Different extraction procedures were applied to the two batches. In the first process the ground roots were extracted exhaustively with petrol at room temperature; the resulting oily extract was fractionated on a column of silica gel and fractions eluted with solvents of increasing polarity. Piperlonguminine (**1**) and corcovadine (**3**) were isolated in this way. With the second batch, extraction was done in a Soxhlet, the extract being chromatographed repeatedly on a series of silica gel columns. By this procedure, all five amides could be isolated. Separation on silica gel proved to be effective, although extremely delicate due to the closeness of the bands. All five amides were eluted with hexane–EtOAc mixtures of composition close to 7:3, thus calling for frequent rechromatography of the fractions. Most difficult to isolate pure was piperovatine (**5**). Its presence was monitored through the procedure by means of its anesthetizing action on the tongue. Several attempts to purify the active fractions were unsuccessful due mainly to their pronounced tendency to darken. The final purification was achieved again through chromatography on a silica gel column and elution with mixtures of hexane and EtOAc. At the end, the compound crystallized after being kept for 11 weeks at −5°.



1 *trans*-2-*trans*-4
2 *cis*-2-*cis*-4



3 *trans*-2-*trans*-4, R = Ac
4 *cis*-2-*cis*-4, R = Ac
6 *trans*-2-*trans*-4, R = H



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The pair piperlonguminine–isopiperlonguminine

Piperlonguminine (**1**), the isobutylamide of *trans,trans*-piperic acid, was originally isolated from the roots of *Piper longum* [1]. Isopiperlonguminine (**2**), $C_{16}H_{19}O_3N$,

*Not to be confused with the genuine jaborandi, comprising several *Pilocarpus* species (family Rutaceae), the source of pilocarpine and other alkaloids.

crystals, mp 140–143°, is herewith described as the corresponding *cis,cis* isomer. The mass spectra of the compounds are identical; IR and UV spectra are similar. The IR spectra differ notably at 985 cm^{-1}, where only **1** shows a strong absorption peak due to the *trans* configuration. Comparison of the UV spectra shows essentially a hypsochromic shift of the main absorption band, of the order of 10 nm. Significant differences appear in the ^1H NMR spectra, where signals in the region of the olefinic protons appear much less complex for **2** than for **1** (see Table 1).

The positions of the signals agree with those reported for synthetic *cis,cis*-piperic acid [2] and for *cis,cis*-piperine (chavicine) [3]. Doubts about the precise assignments for protons B and C [3] could be dispelled with the aid of the 'spin tickling' technique of double resonance. Irradiating the proton corresponding to the most upfield signal (H_A, δ 5.69, *d*, J = 11 Hz) determines splitting of the signal at δ 6.85 (*t*, J = 11 Hz), which must therefore be assigned to H_B. Conversely, irradiation of the most downfield signal (presumably the deshielded H_C, (δ 7.45, *t*, J = 11 Hz) determines splitting of two signals, as would have to be expected, one being due to H_B (already identified) and the other one H_D (δ 6.66, *d*, J = 11 Hz). The observed differences between the spectra of the isomers with respect to the protons β to the amide carbonyl group are consistent in that the uniform shielding in the *trans,trans* structures suffers a deshielding effect in the *cis,cis* structures.

The pair corcovadine-isocorcovadine

Corcovadine (**3**), $C_{18}H_{21}O_5N$, crystals, mp 141–144°, exhibits the typical UV absorptions of a piperic acid derivative (see Experimental). The IR spectrum is similar to that of piperlonguminine with respect to the amide function (ν_{max} 3225 and 1640 cm^{-1}), the *trans* configuration of the olefinic double bonds (1000 cm^{-1}) and the methylenedioxy group (925 cm^{-1}). A second carbonyl function is revealed by a strong absorption at 1710 cm^{-1}, which can be attributed to the presence of an acetoxy group. The ^1H NMR spectrum also very much resembles that of piperlonguminine with respect to the piperic acid moiety (see Table 1). In addition, there are signals for geminal methyl groups (6 H, *s*) at δ 1.46; methylene protons (2 H, *d*, J = 6 Hz) at δ 3.59, coupled with the proton at the nitrogen (1 H, *br.*), at δ 6.23. A single signal (3H, *s*) at δ 2.02 is again indicative of an acetoxy group, which was also confirmed by the mass spectrum: with a fragmentation pattern similar to that of piperlonguminine, it shows an additional significant peak at *m/z* 271 ($M^+ - 60$; 14%), corresponding to the loss of one acetic acid molecule from the molecular ion. Other fragments were typical of amides derived from piperic acid. On the basis of these data, structure **3** was assigned to corcovadine. Chemical confirmation was obtained through alkaline hydrolysis, producing the amide **6**, with a tertiary hydroxyl group, and piperic acid.

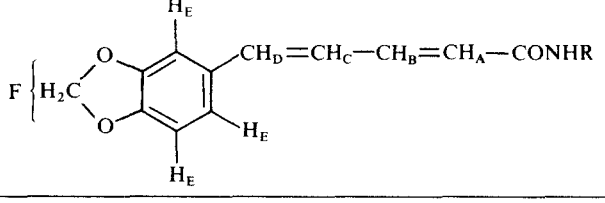
Spectral comparisons analogous to those discussed for piperlonguminine and isopiperlonguminine allowed for correlation of corcovadine (**3**) with its isomer, isocorcovadine (**4**), $C_{18}H_{21}O_5N$, crystals, mp 80–85°. The absence, in the IR spectrum of the latter, of an absorption in the region of 970–990 cm^{-1} points to the absence of double bonds in a *trans* configuration. The *cis* configuration receives support from similarities in the UV and ^1H NMR spectra to those of isopiperlonguminine (**2**) (see Table 1 and Experimental). Mass spectral fragmentations of **3** and **4** are identical.

EXPERIMENTAL

Mps were determined on a Kofler hot stage and are uncorr. UV spectra were obtained in MeOH and IR spectra in KBr unless otherwise stated. ^1H NMR spectra were measured at 100 MHz and MS at 70 eV. Column chromatography was performed on Si gel, and TLC on Si gel H and GF_{254} (Merck). The identity of compounds, whenever stated, was established by direct comparison with authentic samples through mp, mmp, UV, IR, ^1H NMR and MS, as well as TLC.

Plant material. Two batches of *O. corcovadensis* were collected at the hillside of the Corcovado Mountain in Rio de Janeiro, in December, at a 1 year interval.

Table 1. ^1H NMR spectral data of the two pairs of isomeric piperic acid amides from *O. corcovadensis* (100 MHz, solvent $CDCl_3$, chemical shifts in ppm, δ units, J in Hz)



R	Compound	Configuration	A	B	C	D	E	F
CH_2CHMe_2	Piperlonguminine (**1**)	*trans, trans*	5.90 (*d*) J = 15	7.38 (*m*)	—*	—	—	5.94 (*s*)
CH_2CHMe_2	Isopiperlonguminine (**2**)	*cis, cis*	5.69 (*d*) J = 11	6.85 (*t*) J = 11	7.45 (*t*) J = 11	6.66 (*d*) J = 11	6.78 (*s*)	5.96 (*s*)
CH_2CMe_2OAc	Corcovadine (**3**)	*trans,trans*	5.94 (*d*) J = 15	7.40 (*m*)	—	—	—	5.95 (*s*)
CH_2CMe_2OAc	Isocorcovadine (**4**)	*cis,cis*	5.69 (*d*) J = 11	6.91 (*t*) J = 11	7.44 (*t*) J = 11	6.64 (*d*) J = 11	6.76 (*s*)	5.93 (*s*)

*In the *trans,trans* structures individualization of protons C, D and E was not possible at the scale of the spectrum. For positions of these signals see Experimental.

Extraction and separation I. The dried, powdered roots (1600 g) were exhaustively extracted by maceration with petrol. The combined extracts (22 l.) were concd under red. pres. to give a dark green viscous mass (7.70 g). The material was treated with Me_2CO and the soluble portion was again evapd to dryness under red. pres. The dark oily residue (5.86 g) was chromatographed on a Si gel column (350 g) and developed with solvent mixtures of increasing polarity. Toluene–EtOAc (19:1) eluted piperlongumine (**1**, 36 mg). Elution with toluene–EtOAc 9:1 and 4:1 afforded fractions which were combined and submitted to the same procedure. Repeated chromatography followed by five recrystallizations from MeOH yielded corcovadine (**3**, 58 mg). Although these last fractions were strongly anesthetizing to the tongue, separation of the active material could not be achieved.

Extraction and separation II. The dried, powdered roots (1900 g) were extracted with petrol in a Soxhlet until the extract was clear (72 hr.) The filtered extract was concd under red. pres., yielding a dark green viscous oil (22 g). The material was chromatographed on a column of Si gel (300 g) and developed first with hexane, followed by solvent mixtures of increasing polarity, through toluene, EtOAc and MeOH. This procedure resulted in the separation of four crude fractions, of which only one, the one eluted with EtOAc, showed anesthetic activity. The fractions were repeatedly rechromatographed, monitoring the procedure by means of TLC. In this, visualization of all the compounds in UV light was very helpful: the *trans* compounds showed intense, blue fluorescence, while the *cis* compounds were detached from the background as dark spots. Eventually, all five amides were separated in this way. Solvents for crystallization were: toluene for piperlonguminine; hexane–EtOAc (2:1) for isopiperlonguminine; MeOH for corcovadine; hexane–EtOAc (4:1) for isocorcovadine; MeOH for piperovatine. All were obtained as colourless crystals.

Piperlonguminine (**1**). Mp 167–169° (lit. [1] 166–168°). IR ν_{max} cm^{-1}: 3280, 1640, 1615, 1550, 1495, 1470, 1430, 1360, 1240, 1185, 1030, 985, 920, 850. UV λ_{max}^{MeOH} nm (log ε): 342 (4.54), 307 (4.34), 253 (4.17), 245 (4.17). ^{1}H NMR: δ 0.92 (6 H, *d*, *J* = 7.5 Hz), 1.80 (1 H, *m*), 3.16 (2 H, *t*, *J* = 6 Hz), 5.58 (1 H, *br.*), 5.90 (H_A, *d*, *J* = 15 Hz), 5.94 (2 H, *s*), 6.60–6.98 (6 H, *m*), 7.38 (H_B, *m*). MS *m/z* (rel. int.): 273 (M^+, 34), 216 (10), 201 (50), 173 (46), 172 (25), 152 (4), 143 (27), 135 (8), 115 (100), 96 (16). Found: 273.1348; $C_{16}H_{19}O_3N$ requires: 273.1359.

Isopiperlonguminine (**2**). Mp 140–143°. IR ν_{max} cm^{-1}: 3250, 2875, 1640, 1620, 1605, 1550, 1500, 1485, 1435, 1263, 1250, 1040, 920, 825. UV λ_{max}^{MeOH} nm (log ε): 332 (4.51), 312 (4.40), 255 (4.33), 245 (4.33). ^{1}H NMR: δ 0.95 (6 H, *d*, *J* = 7 Hz), 1.80 (1 H, *m*), 3.16 (2 H, *t*, *J* = 7 Hz), 5.69 (H_A, *d*, *J* = 11 Hz and NH, *br.*), 5.96 (2 H, *s*), 6.66 (H_D, *d*, *J* = 11 Hz), 6.78 (3 H, *s*), 6.85 (H_B, *t*, *J* = 11 Hz), 7.45 (H_C, *t*, *J* = 11 Hz). MS *m/z* (rel. int.): 273 (M^+, 30), 216 (3), 201 (60), 173 (50), 172 (20), 152 (5), 143 (27), 135 (12), 115 (100), 96 (25). Found: 273.1336; $C_{16}H_{19}O_3N$ requires: 273.1359.

Corcovadine (**3**). Mp 141–144°. IR ν_{max} cm^{-1}: 3225, 1710, 1640, 1585, 1540, 1470, 1430, 1350, 1315, 1235, 1190, 1125, 1030, 1000, 925, 870, 800. UV λ_{max}^{MeOH} nm (log ε): 342 (4.65), 307 (4.40), 252 (4.19), 244 (4.19). ^{1}H NMR: δ 1.46 (6 H, *s*), 2.02 (3 H, *s*), 3.59 (2 H, *d*, *J* = 6 Hz), 5.94 (H_A, *d*, *J* = 15 Hz), 5.95 (2 H, *s*), 6.23 (1 H, *br.*), 6.64–6.95 (3 H arom., 2 H olef., *m*), 7.40 (H_B, *m*). MS *m/z* (rel. int.): 331 (M^+, 46), 271 (14), 201 (100), 173 (26), 172 (30), 143 (17), 135 (15), 115 (54), 43 (60). Found: C, 65.41; H, 6.14; N, 4.38. $C_{18}H_{21}O_5N$ requires: C, 65.25; H, 6.34; N, 4.22%.

Isocorcovadine (**4**). Mp 80–85°. IR ν_{max} cm^{-1}: 3230, 1710, 1615, 1600, 1565, 1540, 1490, 1435, 1365, 1280, 1240, 1205, 1125, 1030, 935, 920, 860, 815. UV λ_{max}^{MeOH} nm (log ε): 327 (4.48), 310 (4.38), 254 (4.41), 245 (4.39). ^{1}H NMR: δ 1.45 (6 H, *s*), 1.99 (3 H, *s*), 3.56 (2 H, *d*, *J* = 6 Hz), 5.69 (H_A, *d*, *J* = 11 Hz), 5.93 (2 H, *s*), 6.22 (1 H, *br.*), 6.64 (H_D, *d*, *J* = 11 Hz), 6.76 (3 H, *s*), 6.91 (H_B, *t*, *J* = 11 Hz), 7.44 (H_C, *t*, *J* = 11 Hz). MS *m/z* (rel. int.): 331 (M^+, 20), 271 (15), 201 (100), 173 (38), 172 (27), 143 (15), 135 (16), 115 (43), 43 (43). Found: 331.1491; $C_{18}H_{21}O_5N$ requires: 331.1413.

Piperovatine (**5**). Mp 115–119° (lit. [4] 120–121°). IR ν_{max} cm^{-1}: 3225, 2880, 1660, 1625, 1620, 1545, 1510, 1245, 1180, 1035, 995, 820. UV λ_{max}^{MeOH} nm (log ε): 263 (4.42). ^{1}H NMR: δ 0.93 (6 H, *d*, *J* = 7 Hz), 3.17 (2 H, *t*, *J* = 6 Hz), 3.43 (2 H, *d*, *J* = 5 Hz), 3.80 (3 H, *s*), 5.77 (1 H, *d*, *J* = 15 Hz), 5.92–6.40 (3 H, *m*), 6.96 (4 H, *dd*, *J* = 8 Hz). MS *m/z* (rel. int.): 273 (M^+, 41), 201 (12), 173 (100), 172 (21), 159 (24), 158 (30), 152 (82), 139 (63), 121 (44), 100 (27), 96 (55). Found: 273.1707; $C_{17}H_{23}O_2N$ requires: 273.1722.

Alkaline hydrolysis of corcovadine. Corcovadine (**3**) (34 mg) was dissolved in 20 ml 0.1 ethanolic KOH with slight heating (40°), followed by boiling under reflux for 1 hr. By this time the starting material had disappeared, as verified by TLC (blue fluorescent spots under UV light of both the starting material and products). After adding H_2O (30 ml), the mixture was acidified with HCl and extracted 5 × with 50 ml Et_2O. The Et_2O layer was dried, filtered and evapd. The residue (32 mg) was worked up on a column of Si gel, developing with solvent mixtures of increasing polarity. Hexane–EtOAc (3:2) eluted piperic acid, mp 210–215°, identified by comparison with an authentic sample. A second product (**6**, 25 mg), eluted with hexane–EtOAc (2:3), was obtained in the form of a glass, resisting all attempts of crystallization.

Piperic acid N-(*2-hydroxy-isobutyl*)-*amide* (**6**). Light greenish, glassy scales. IR ν_{max}^{film} cm^{-1}: 3330, 2985, 2900, 1640, 1600, 1550, 1495, 1440, 1280, 1240, 1175, 1095, 1040, 990, 930, 820. UV λ_{max}^{EtOH} nm (log ε): 341 (4.58), 310 (4.43), 255 (4.43), 251 (4.40). ^{1}H NMR: 1.25 (6 H, *s*), 3.35 (2 H, *d*, *J* = 6 Hz), 5.95 (2 H, *s*), 5.96 (1 H, *d*, *J* = 15 Hz), 6.62–6.93 (5 H, *m*), 7.35 (1 H, *m*). MS *m/z* (rel. int.): 289 (M^+, 60), 271 (5), 230 (43), 201 (100), 173 (42), 172 (32), 143 (24), 135 (23), 115 (83), 96 (20), 89 (20), 59 (43). Found: 289.1368; $C_{16}H_{19}O_4N$ requires: 289.1308.

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