Panurensine and Norpanurensine, New Bisbenzylisoquinoline Alkaloids from *Abuta panurensis*

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Panurensine and norpanurensine, new bisbenzylisoquinoline alkaloids from *Abuta panurensis* Eichler, have been assigned structures 1 and 2, respectively, on the basis of spectroscopic and chemical evidence. They are the first examples of bisbenzylisoquinolines containing both a 5–7′ and an 11–12′ ether bridge.

Many plants of the family Menispermaceae have been shown to be a rich source of alkaloids of the benzylisoquinoline and benzylisoquinoline-derived types.[1] As part of a broad search for new anticancer alkaloids, we are studying a number of previously unexamined South American members of this family, especially species of the genus *Abuta*.[2] The Amazonian species, *Abuta panurensis*, has now been found to contain an alkaloid fraction exhibiting marked activity in the KB-nasopharynx tumor cell system. We wish to report here the isolation and structure determination of the two major constituents of this fraction, namely the new bisbenzylisoquinoline alkaloids panurensine (1) and norpanurensine (2).

Panurensine (1) was obtained as white needles, mp 156–158°, which readily become yellow on exposure to light and air. The composition $C_{37}H_{40}N_2O_6$ was determined by high-resolution mass spectrometry.

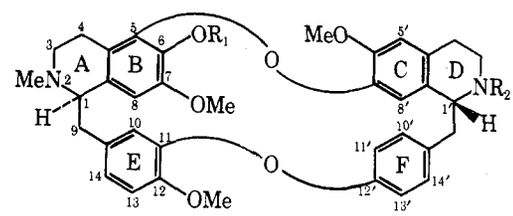
The infrared spectrum (KBr) of panurensine showed the absence of a carbonyl band, but a band at 3400 cm^{-1}, attributable to a nonassociated phenolic group, was observed.

The NMR spectrum of panurensine showed the presence of three aromatic methoxyls at δ 3.46, 3.82 and 3.92, as well as two methylimino groups at δ 2.40 and 2.55. Of the ten aromatic protons present, four were clearly discernible as singlets at δ 6.61, 5.82, 5.24, and 5.02. The high degree of shielding of the latter three protons certainly is the most striking feature of the spectrum.

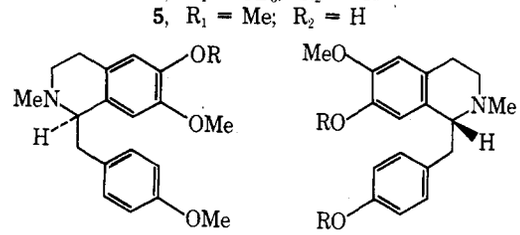
Treatment of panurensine with excess diazomethane afforded *O*-methylpanurensine (3), confirming the presence of one phenolic function in the parent alkaloid. The corresponding reaction of panurensine with deuteriodiazomethane in dioxane–deuterium oxide[3] yielded the corresponding *O*-trideuteriomethyl derivative 4. A comparison of the NMR spectra of 3 and 4 showed that the new methyl of the methyl ether 3 is represented by the normal methoxyl signal at δ 3.75. This result rules out the possibility that panurensine may be a normal head-to-head dimer having a C-7 hydroxyl, since a methoxyl at the corresponding position would be highly shielded.[4]

The mass spectrum of panurensine is typical of that of a bisbenzylisoquinoline alkaloid containing both head-to-head and tail-to-tail ether bridges.[5] Thus, weak peaks at M − 107, M − 121, and M − 137 occur in the spectra of both panurensine (1) and its methyl ether 3. These peaks are characteristic of a tail-to-tail diphenyl ether system containing one methoxyl substituent, and require that the phenolic group of 1 be located on one of the isoquinoline units. In accord with this general formulation, the base peak of panurensine, representing the linked isoquinoline units, is seen at *m/e* 381, a value which is shifted to 395 in the spectrum of methyl ether 3.

The mass spectrum of the trideuteriomethyl ether 4 further defines the environment of the original phenolic hydroxyl of 1. Loss of the CD_3 group from the head-to-head

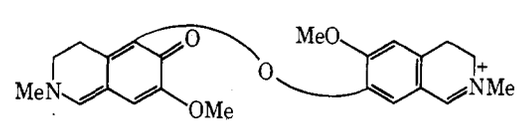


1, R_1 = H; R_2 = Me
2, R_1 = R_2 = H
3, R_1 = R_2 = Me
4, R_1 = CD_3; R_2 = Me
5, R_1 = Me; R_2 = H



6, R = CD_3
7, R = Me

8, R = H
9, R = Et



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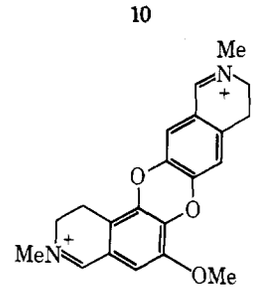

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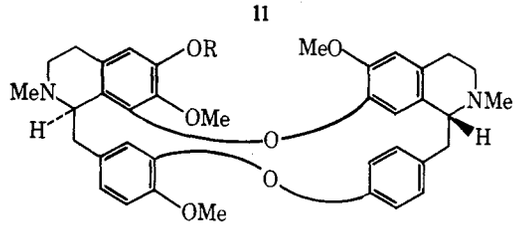

12, R = H
13, R = Me

fragment of the molecule gives rise to an ion (10) at *m/e* 380, consistent with the loss of this group from a C-6 (or C-6′) position to give a stabilized *p*-quinonoid species. Furthermore, the same intense doubly charged dioxane fragment at *m/e* 175 (11) appears in the mass spectra of both the methyl ether 3 and the trideuteriomethyl analog 4, in-

dicative of the presence of an *o*-hydroxy-*o'*-methoxydiphenyl ether system in the top portion of panurensine itself.

Treatment of *O*-methylpanurensine (**3**) with sodium in liquid ammonia cleanly cleaved the molecule into nonphenolic and phenolic portions.[6] The nonphenolic product was identical with authentic (*R*)-*O*-methylarmepavine (**7**). The phenolic product was identified as (*R*)-*N*-methylcoclaurine (**8**) by comparison with an authentic sample of its enantiomer and by conversion to the known crystalline oxalate[7] of its *O,O*-diethyl ether (**9**). A similar reductive cleavage of *O*-trideuteriomethylpanurensine (**4**) afforded (*R*)-*N*-methylcoclaurine (**8**) and the deuterated nonphenolic base **6**. A comparison of the mass spectrum of **6** with that of (*R*)-*O*-methylarmepavine (**7**) established that the CD_3 group was in the isoquinoline unit (*m/e* 209 for the base peak), while a comparison of their NMR spectra showed that the deuterated compound still possessed the more shielded C-7 methoxyl group.

The above data are consistent only with two possible structures (**1** and **12**) for panurensine. Structure **12** is clearly ruled out, since its *O*-methyl derivative would be *l*-tetrandrine (phaeanthine, **13**), and the reported NMR values for tetrandrine[4] clearly distinguish it from *O*-methylpanurensine. Panurensine must therefore be assigned structure **1**.

The three highly shielded aromatic protons appearing in the NMR spectrum of panurensine are clearly assignable to protons at C-8, C-8', and C-10, since molecular models reveal these protons to lie over the centers of nearby aromatic rings. Acid-catalyzed deuteration[8] of *O*-methylpanurensine introduces two deuteriums into the molecule, neither deuterium having replaced one of the shielded aromatic protons. The mass spectrum of the deuterated product showed peaks indicative of one deuterium in ring C (*m/e* 175 and 191) and one in ring E (*m/e* 486); the deuterated positions must therefore be C-5' and C-13.

Thalmine (*S,S*), lauberine (*S,R*), dryadine (*R,S*), and dryadodaphnine (*R,S*) belong to a small group of bisbenzylisoquinoline alkaloids which arise biogenetically by the oxidative coupling of one coclaurine and one isococlaurine unit, all have a 5–7' ether bridge at the top and a 12–11' ether bridge at the bottom of the molecule.[9] The most shielded aromatic protons of this group appear only slightly upfield (δ 5.88–6.25) from the normal aromatic region.[10] Panurensine is biogenetically related to these alkaloids, but is the first example of a bisbenzylisoquinoline having 5–7' and 11–12' ether bridges. This structural feature is apparently responsible for the unusually high shielded (δ 5.02–5.82) aromatic protons of panurensine, a characteristic which should be of diagnostic value in the identification of further related alkaloids which may be isolated in the future.

Norpanurensine (**2**), mp 175°, formed white crystals from methanol, and had the empirical composition $C_{36}H_{38}N_2O_6$. N-Methylation of **2** with formalin and sodium borohydride afforded panurensine (**1**). The location of the secondary amine function was revealed by a study of the mass spectra of norpanurensine and its *O*-methyl derivative (**5**), both of which showed peaks at *m/e* 160 and 176, corresponding to the loss of the CD isoquinoline unit containing the unmethylated nitrogen. Structure **2** may therefore be unambiguously assigned to norpanurensine.

Experimental Section

Melting points are uncorrected. NMR spectra were determined in $CDCl_3$ solution with tetramethylsilane as internal standard using Varian A-60, HR-100, or 220-MHz instruments. Infrared, ultraviolet (ethanol solution), mass spectra and optical rotations (chloroform solution at room temperature) were determined using Perkin-Elmer Models 137, 202, 270, and 141 instruments, respectively. All preparative chromatography (PLC) was carried out on silica plates using 10:1 $CHCl_3$–MeOH (developer A) or 20:1 $CHCl_3$–MeOH (developer B). *Abuta panurensis* (*Prance 14973*) was collected by G. T. Prance on the Rio Cuieras basin of the lower Rio Negro, Amazonas, Brazil, and identified by B. A. Krukoff. A voucher specimen has been deposited at New York Botanical Garden and other institutions.

Isolation of Panurensine (1) and Norpanurensine (2) from *Abuta panurensis* Eichler. Ground stems of liana (1.67 kg) were extracted exhaustively first with aqueous ammonia–ether, and then with 4 *N* hydrochloric acid. The acid extract was basified with ammonia and extracted with ether. The combined ether extracts yielded 17.21 g of crude residue consisting of neutral and basic material. This material was subjected to gradient pH-countercurrent distribution (100 transfers) between chloroform and aqueous acid, starting with pH 6.5 citrate–phosphate buffer and ending with 3 *M* phosphoric acid. The acidic aqueous layers from tubes 28–41 and 48–61 yielded, upon basification and reextraction with chloroform, 2.2 and 3.3 g, respectively of crude norpanurensine (**2**) and panurensine (**1**). Panurensine crystallized from methylene chloride–hexane to yield pale yellow crystals, mp 155°. Further purification via PLC and recrystallization from methylene chloride afforded colorless crystals: mp 156–158°; $[\alpha]_D$ − 245.6° (*c* 0.5); ir (KBr) 3400 cm^{-1} (OH); uv λ_{max} (ε) 225 nm (24,000), 238 (35,400), 284 (16,800); λ_{max} (EtOH–NaOH) (ε) 225 nm (25,500), 240 (39,000), 288 (20,700); NMR (220 MHz) δ 2.40, 2.55 (s, 3 H each, 2 NMe), 3.46, 3.82, 3.92 (s, 3 H each, 3 OMe), 5.02 (s, 1 H), 5.24 (s, 1 H), 5.82 (s, 1 H), 6.61 (s, 1 H) (m, 6.42–7.26); mass spectrum *m/e* (rel intensity) 608 (M^+, 73), 607 (43), 501 (<1), 487 (1), 471 (2), 381 (100), 192 (7), 191 (78), 190 (16), 176 (10), 174 (15), 168 (17); high-resolution mass spectrum *m/e* 608.29067 ($C_{37}H_{40}N_2O_6$ requires *m/e* 608.28863).

Norpanurensine crystallized from methanol as needles: mp 175°; $[\alpha]_D$ −250° (*c* 0.1); ir (KBr) 3400 (OH) and 3200 cm^{-1} (NH); uv λ_{max} (ε) 223 nm (11,000), 240 (20,000), 288 (12,600); NMR δ 2.42 (s, 3 H, NMe), 3.47, 3.83, 3.94 (s, 3 H each, 3 OMe), 5.08 (s, 1 H), 5.28 (s, 1 H), 6.08 (s, 1 H), 6.63 (s, 1 H), 6.50–7.24 (m, 6 H); mass spectrum *m/e* (rel intensity) 594 (M^+, 26), 593 (11), 487 (<1), 473 (1), 457 (2), 367 (100), 206 (11), 205 (15), 192 (8), 191 (10), 190 (23), 184 (92), 176 (14), 168 (5), 161 (26), 160 (7); high-resolution mass spectrum *m/e* 594.27120 ($C_{36}H_{38}N_2O_6$ requires 594.27298).

***O*-Methylpanurensine (3).** To a solution of **1** in methanol was added ethereal diazomethane in two portions during 2 days. The mixture was left in the dark. The usual work-up gave **3** as colorless prisms from EtOAc–hexane: mp 124–125°; $[\alpha]_D$ −210° (*c* 0.05); NMR δ 2.48, 2.60 (s, 3 H each, 2 NMe), 3.44, 3.75, 3.93, 3.96 (s, 3 H each, 4 OMe), 5.15 (s, 1 H), 5.32 (s, 1 H), 5.82 (s, 1 H), 6.73 (s, 1 H), 6.40–7.31 (m, 6 H); mass spectrum *m/e* (rel intensity) 622 (M^+, 100), 621 (75), 515 (<1), 501 (1), 485 (2), 395 (74), 220 (4), 206 (6), 198 (96), 190 (22), 175 (81), 174 (15); high-resolution mass spectrum *m/e* 622.3075 ($C_{38}H_{42}N_2O_6$ requires 622.3042).

***O*-Trideuteriomethylpanurensine (4).** To a cooled solution of excess diazomethane in dioxane[3] (10 ml) and D_2O (1 ml) was added a solution of **1** (50 mg) in dioxane (2 ml) and D_2O (1 ml). After standing for 24 hr in the dark, the usual work-up afforded **4** as an oil (40 mg): NMR δ 2.47, 2.59 (s, 3 H each, 2 NMe), 3.44, 3.92, 3.94 (s, 3 H each, 3 OMe), 5.15 (s, 1 H), 5.32 (s, 1 H), 5.82 (s, 1 H), 6.72 (s, 1 H), and 6.50–7.21 (m, 6 H).

Acid-Catalyzed Deuteration[8] of 3. A solution of **3** (40 mg) in 3% DCl–D_2O (1.5 ml) was heated at 110° under nitrogen in a sealed tube. After 120 hr, the reaction mixture was worked up as usual to give the 5',13-dideuterio derivative as a pale yellow oil (36 mg): NMR δ 2.46, 2.58 (s, 3 H each, 2 NMe), 3.43, 3.74, 3.90, 3.93 (s, 3 H each, 4 OMe), 5.14 (s, 1 H), 5.32 (s, 1 H), 5.82 (s, 1 H), 6.71 (s, <1 H), 6.50–7.30 (5–6 H); mass spectrum *m/e* (rel intensity) 624 (M^+, 57), 623 (67), 622 (41), 517 (M − 107, <1), 486 (M − 138, 2), 386 (56), 198.5 (100), 193 (4), 192 (6), 191 (9), 176 (20).

Sodium–Ammonia Cleavage of 4. To liquid ammonia (400 ml) at −78° was added alternately, with stirring, small pieces of sodium (total of 1 g) and portions of a solution of **4** (400 mg) in dry dioxane, making sure that the color remained blue, prior to each addition of the alkaloid solution. Finally some extra pieces of sodium were added until the blue color persisted for 15 min. The ammonia was then allowed to evaporate overnight. The residue was extracted into methanol. The residue from the methanol was dissolved in water and extracted with ether to separate the nonphenolic fraction (130 mg).

From the aqueous fraction, after saturation with ammonium

chloride (pH 8–9) and extraction with ether (addition of a little $NaBH_4$ retarded air oxidation) was obtained the phenolic fraction (98 mg).

From the nonphenolic fraction, **6** was isolated by PLC (developer B) as an oil: $[\alpha]D$ −76° (*c* 0.075); NMR δ 2.53 (s, 3 H, NMe), 3.57, 3.77 (s, 3 H each, 2 OMe), 6.07 (s, 1 H), 6.58 (s, 1 H), 6.80 (d, 2 H, *J* = 8 Hz), 7.05 (d, 2 H, *J* = 8 Hz); mass spectrum *m/e* (rel intensity) 330 (M^+, 3), 329 (6), 209 (100), 121 (15). The oxalate of **6** crystallized from ethanol–ether, mp 124–125°, $[\alpha]D$ −70° (*c* 0.11).

From the phenolic fraction was obtained the amorphous **8**: $[\alpha]D$ −25.2° (*c* 0.21); NMR δ 2.42 (s, 3 H, NMe), 3.77 (s, 3 H, OMe), 5.90 (broad, s, 2 H, 2 OH), 6.52 (s, 1 H), 6.29 (s, 1 H), 6.60 (d, 2 H, *J* = 8 Hz), 6.90 (d, 2 H, *J* = 8 Hz); mass spectrum *m/e* (rel intensity) 299 (M^+, 2), 192 (100), 107 (9). The ir ($CHCl_3$) and NMR spectra of **8** were identical with those of an authentic sample of its enantiomer.

A portion of the phenolic fraction (25 mg) was treated with ethereal diazoethane. Work-up in the usual manner after two days yielded *O,O'*-diethyl-*N*-methylcoclaurine (**9**) (20 mg) as a pale yellow oil: NMR 1.31 (t, 3 H, *J* = 7 Hz), 1.38 (t, 3 H, *J* = 7 Hz), 2.53 (s, 3 H, NMe), 3.81 (s, 3 H, OMe), 3.68 (q, 2 H, *J* = 7 Hz), 3.98 (q, 2 H, *J* = 7 Hz), 6.13 (s, 1 H), 6.58 (s, 1 H), 6.74 (d, 2 H, *J* = 8 Hz), 7.00 (d, 2 H, *J* = 8 Hz). It was converted to the oxalate which crystallized from ethanol–ether as needles, mp 173–175°,[7] $[\alpha]D$ −115° (*c* 0.16).

Anal. Calcd for $C_{24}H_{31}O_7N$: C, 64.71; H, 6.96; N, 3.14. Found: C, 64.87; H, 7.22; N, 3.13.

Sodium–Ammonia Cleavage of 3. The sodium–liquid ammonia cleavage was carried out on **3** (200 mg) exactly as described earlier and the products were separated into nonphenolic and phenolic fractions. From the nonphenolic fraction, **7** was obtained after PLC (developer A) as a colorless oil (50 mg), NMR 2.52 (s, 3 H, NMe), 3.58, 3.77, 3.83 (s, 3 H each, 3 OMe), 6.08 (s, 1 H), 6.57 (s, 1 H), 6.80 (d, 2 H, *J* = 8 Hz), 7.05 (d, 2 H, *J* = 8 Hz); these values are identical with those reported for *O*-methylarmepavine.[11] The oxalate was obtained as needles from ethanol, mp 112°, $[\alpha]D$ −98° (*c* 0.061) (lit.[7] mp 112°), and found to be identical (ir, melting point, and $[\alpha]D$) with an authentic sample prepared from the *R* enantiomer of *O*-methylarmepavine. Work-up of the phenolic fraction afforded **8**.

N-Methylation of 2. To a solution of **2** (45 mg) in $CHCl_3$ (4 ml)–MeOH (4 ml) was added formalin with stirring. After 1 hr, the mixture was cooled in ice and treated with excess sodium borohydride in small portions. After stirring for an additional 1 hr, the solvent was removed and the residue was treated with water. The product was extracted into chloroform and purified via PLC (developer A) to give a product (35 mg), mp 156–158° from methylene chloride–hexane, $[\alpha]D$ −245° (*c* 0.5), identical in all respects (ir, melting point, mixture melting point, rotation, and mass spectra) with panurensine (**1**).

***O*-Methylnorpanurensine (5).** Treatment of **2** (50 mg) with ethereal diazomethane for 2 days in the dark followed by the usual work-up gave an oily residue (45 mg). Purification by PLC (developer A) gave **5** as an oil: NMR 2.45 (s, 3 H, NMe), 3.47, 3.77, 3.94, 3.97 (s, 3 H each, 4 OMe), 5.18 (s, 1 H), 5.31 (s, 1 H), 6.05 (s, 1 H), 6.72 (s, 1 H), 6.52–7.38 (m, 6 H); mass spectrum *m/e* (rel intensity) 6.08 (M^+, 65), 607 (28), 501 (1), 487 (2), 471 (3), 381 (97), 206 (9), 205 (18), 204 (22), 191 (100), 183 (13), 176 (6), 175 (10), 168 (37), 161 (10), 160 (7); high-resolution mass spectrum, *m/e* 608.28838 ($C_{37}H_{40}N_2O_6$ requires 608.28863).

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Registry No.—1, 55701-99-0; **2,** 55702-00-6; **3,** 55702-01-7; **4,** 55722-70-8; **5,** 55702-02-8; **6,** 55702-03-9; **6** oxalate, 55702-04-0; **7,** 5701-00-8; **7** oxalate, 55702-05-1; **8,** 5096-70-8; **9,** 6681-71-6; **9** oxalate, 55723-12-1; **5'**,13-dideuterio-*o*-methylpanurensine, 55722-71-9.

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