(8) We thank Dr. J. C. Orr (Harvard University) for kindly drawing our attention to ref 9, and also for an authentic sample of compound **3a** made by that procedure.
(9) A. Bowers and O. Halpern, U.S. Patent 3,159,621 (Dec 1, 1964); U.S. Patent 3,261,830 (July 19, 1966).
(10) P. Crabbé, "ORD and CD in Chemistry and Biochemistry", Academic Press, New York, N.Y., 1972, p 40.
(11) P. Morand and M. Kaufman, *Can. J. Chem.*, **49**, 3185 (1971).
(12) P. Chamberlain, M. L. Roberts, and G. H. Whitham, *J. Chem. Soc. B*, 1374 (1970), and references cited therein.
(13) We thank Dr. Ira Levin (NIH) for the Raman spectrum, measured using a Cary 81 Raman spectrophotometer. The excitation source was an Argon laser, 800 mW power at 5145 Å.
(14) E. B. Hershberg, E. P. Oliveto, and R. Rausser, *Chem. Ind. (London)*, 1477 (1958).
(15) R. Henderson and C. H. Robinson, *J. Org. Chem.*, **37**, 565 (1972).

Krukovine, a New Bisbenzylisoquinoline Alkaloid from *Abuta splendida* Krukoff and Moldenke

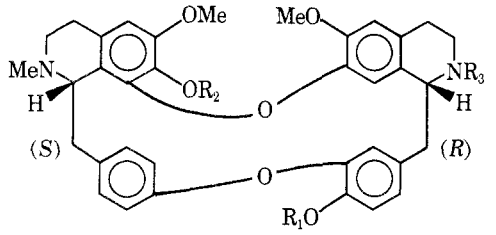
José M. Saá, M. V. Lakshmikantham, Michael J. Mitchell, and Michael P. Cava*

Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19174

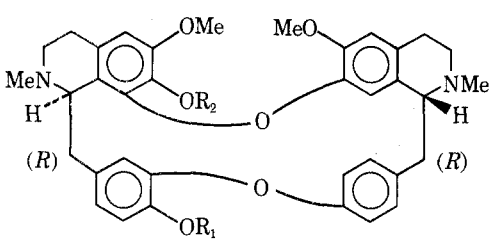
Received July 22, 1975

The bisbenzylisoquinoline alkaloids aromoline, homoaromoline, and krukovine have been found to be the major components of *Abuta splendida* Krukoff and Moldenke. Krukovine has been assigned structure **4** on the basis of both spectroscopic evidence and chemical degradation.

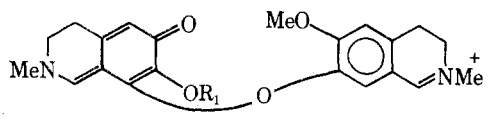
In a continuing search for natural anticancer agents, we have been studying some hitherto unexamined sources of bisbenzylisoquinoline alkaloids, in particular, plants of the genus *Abuta* (*Menispermaceae*).[1] We now wish to report the isolation of the major alkaloidal constituents of the Amazonian species, *Abuta splendida*.[2,3] These include the known bisbenzylisoquinolines aromoline (**1**) and homoaromoline (**2**) as well as the new alkalodd krukovine, which has been shown to have structure **4**.



1, $R_1 = R_2 = H$; $R_3 = Me$
2, $R_1 = Me$; $R_2 = H$; $R_3 = Me$
3, $R_1 = R_2 = R_3 = H$



4, $R_1 = R_2 = H$
5, $R_1 = R_2 = Me$
6, $R_1 = R_2 = CD_3$



7, $R_1 = CD_3$

Aromoline (**1**)[4] was obtained as tiny, colorless prisms, mp 182–183°. Positive identification of this alkaloid was made by direct comparison (ir, mixture melting point, TLC) with an authentic sample obtained by the N-methylation of natural daphnoline (**3**).

Homoaromoline (**2**)[5] was obtained as tiny, white needles, mp 236–237°. It was found to be identical (TLC, mixture melting point) with an authentic sample.

Krukovine (**4**) crystallized from chloroform as colorless prisms of the chloroform solvate, mp 182–183°. The composition $C_{36}H_{38}O_6N_2$ was determined by high-resolution mass spectrometry.

The infrared spectrum of krukovine showed a band at 3400 cm^{-1}, attributable to a nonassociated phenolic group. The NMR spectrum of krukovine showed the presence of two aromatic methoxyls at δ 3.30 and 3.73, as well as two methylimino groups at δ 2.28 and 2.58. Of the ten aromatic protons present five were clearly discernible, one as a singlet at δ 5.97 and four as a pair of doublets at δ 7.11 (d, J = 8 Hz, 2 H) and 7.32 (d, J = 8 Hz, 2 H).

Treatment of krukovine with excess diazomethane, followed by crystallization from acetone, afforded *O,O*-dimethylkrukovine (**5**), mp 125°, confirming the presence of two phenolic functions in the parent molecule. The composition $C_{38}H_{42}O_6N_2$ was determined by high-resolution mass spectroscopy. The corresponding reaction of krukovine with deuteriodiazomethane in dioxane–deuterium oxide[6] yielded the corresponding *O,O*-bistrideuteriomethyl derivative (**6**). A comparison of the NMR spectra of **5** and **6** showed that the new methyl groups of the dimethyl ether **5** are represented by signals at δ 3.18 and 3.93. These values can be assigned to the C-7 and C-12 aromatic methoxyls of a normal head-to-head dimer, since it has been pointed out that a methoxyl at C-7 is highly shielded, while a methoxyl at C-12 (or C-12′) appears in the usual range.[7]

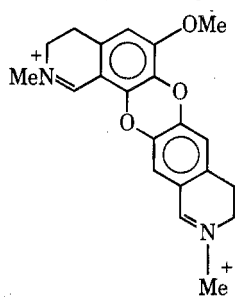
The mass spectrum of krukovine is typical of that of a bisbenzylisoquinoline alkaloid containing both head-to-head and tail-to-tail ether bridges.[8] Thus, a weak peak at M − 107 occurs in the spectra of krukovine (**4**), its dimethyl ether **5**, and the deuterated dimethyl ether **6**. Furthermore, a weak peak at M − 137 is observed in the spectrum of the dimethyl ether **5**, which is shifted to M − 140 in the spectrum of the corresponding deuterated derivative **6**, whereas this feature is absent in the spectrum of krukovine itself. These latter peaks are characteristic of a tail-to-tail diphenyl ether system containing one methoxyl substituent. This requires that one of the phenolic groups of **4** be located on the tail-to-tail diphenyl ether system, and that the second phenolic group of **4** be located on one of the isoquinoline units. In accord with this general formulation, the very strong peak of krukovine representing the linked isoquino-

line units is seen at *m/e* 381, a value which is shifted to *m/e* 395 in the spectrum of dimethyl ether **5**, and to *m/e* 398 in the spectrum of the deuterated dimethyl ether **6**.

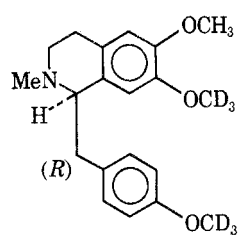
The mass spectrum of the *O,O*-bistrideuteriomethyl ether (**6**) further defines the environment of the original phenolic hydroxyl of **4** located on the linked isoquinoline units. Loss of CH_3 from the head-to-head fragment of the molecule gives rise to an ion **7** at *m/e* 384, consistent with the loss of this group from a C-6 (or C-6′) position to give a stabilized *p*-quinonoid species. Furthermore, the same doubly charged dioxane fragment at *m/e* 175 (**8**) appears in the mass spectra of both the dimethyl ether **5** and the bistrideuteriomethyl analogue (**6**), indicative of the presence of an *o*-hydroxy-*o*′-methoxydiphenyl ether system in the top portion of krukovine itself.

The above data, as well as the negative rotation of dimethylkrukovine (**5**), suggested that the latter might be identical with phaeanthine,[9] despite the far higher melting point (216–217°, ether) reported for the latter. Indeed, crystallization of authentic high-melting phaeanthine from acetone afforded the low-melting form, identical with *O,O*-dimethylkrukovine.

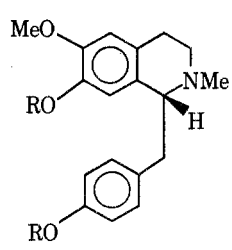
Treatment of *O,O*-bis(trideuteriomethyl)krukovine (**6**) with sodium in liquid ammonia cleanly cleaved the molecule into nonphenolic and phenolic portions.[10] The nonphenolic product was identical with an authentic sample of (*R*)-*O,O*-bis(trideuteriomethyl)-*N*-methylcoclaurine (**9**). The phenolic product was identified as (*R*)-*N*-methylcoclaurine (**10**) by comparison with an authentic sample of its enantiomer and by conversion to the known crystalline oxalate[11] of its *O,O*-diethyl ether (**11**). These observations are



8



9



10, R = H
11, R = Et

entirely consistent with structure **4** for krukovine as the enantiomer of atherospermoline[12] (**4**, *SS*).

Experimental Section

Melting points are uncorrected. NMR spectra were determined in $CDCl_3$ solution (unless otherwise indicated) with tetramethylsilane as internal standard using Varian A-60, HR-100, and Jeol instruments. Infrared (KBr), ultraviolet (ethanol solution), mass spectra, and optical rotations (chloroform solutions 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 20:1 $CHCl_3$–MeOH. *Abuta splendida* (Schunke 1971/38) was collected by J. Schunke in Mariscal Caceres, Tocache Nuevo, Department of San Martin, Peru, and identified by B. A. Krukoff. A voucher specimen has been deposited at the New York Botanical Garden and other institutions.

Isolation of Aromoline (1), Homoaromoline (2), and Krukovine (4) from *Abuta splendida* Krukoff and Moldenke. Stems of liana (4.86 kg) were exhaustively extracted first with aqueous ammonia–ether and then with 2 *N* HCl. The combined ether extracts yielded 43.5 g of crude residue consisting of neutral and basic material. This material was subjected to gradient pH countercurrent distribution (200 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 were basified, reextracted with chloroform, and divided into several fractions: A (tubes 1–16), B (tubes 17–25), C (tubes 26–36), D (tubes 37–42), E (tubes 43–55), F (tubes 56–66), G (tubes 67–104), H (tubes 105–122).

Fraction B (4.64 g) crystallized from chloroform to give colorless prisms (1.84 g) of krukovine (**4**): mp 182–183°; $[\alpha]_D$ −180° (*c* 0.06); ir (KBr) 3400 cm^{-1} (OH); uv λ_{max} (ϵ) 285 (6600); λ_{min} 260 (2850); λ_{max} (NaOH) 291 (9430); λ_{min} (NaOH) 272 (7540); NMR ($CDCl_3$ + Me_2SO-d_6) δ 2.28, 2.58 (s, 3 H each, 2 NMe), 3.30, 3.73 (s, 3 H each, 2 OMe), 5.97 (s, 1 H), 7.11 (d, J = 8 Hz, 2 H), 7.32 (d, J = 8 Hz, 2 H) (m, 6.28–6.75); mass spectrum *m/e* (rel intensity) 594 (M^+, 69), 593 (44), 487 (<1), 403 (14), 381 (100), 192 (75), 191 (95), 190 (24), 174 (32), 168 (25); high-resolution mass spectrum *m/e* 594.27492 (calcd for $C_{36}H_{38}O_6N_2$, 594.27298). Solvent-free **4** showed $[\alpha]_D$ −201°, indicating a $CHCl_3$ content of ca. 10% (0.5 molar equiv) for the crystalline solvate.

Crystallization ($CHCl_3$) of the mother liquors of fraction B yielded aromoline (**1**, 2.1 g) as colorless prisms, mp 182–183°, $[\alpha]_D$ +235°. Two further recrystallizations from $CHCl_3$ gave crystals: mp 182–183; $[\alpha]_D$ +320° (*c* 0.05) (lit.[4] mp 175°, $[\alpha]_D$ + 327°); NMR δ 2.48, 2.55 (s, 3 H each, 2 NMe), 3.57, 3.79 (s, 3 H each, 2 OMe), 5.63 (s, 1 H) (m, 6.39–7.69); mass spectrum *m/e* (rel intensity) 594 (M^+, 41), 593 (34), 487 (4), 403 (6), 381 (90), 192 (30), 191 (100), 190 (18), 175 (18), 174 (35), 168 (40).

Fraction C (6.56 g) crystallized from $CHCl_3$ to give aromoline (5.15 g).

Fraction F (0.18 g) crystallized from acetone to give white needles of homoaromoline (**2**, 0.10 g): mp 236–237°; $[\alpha]_D$ +371° (*c* 0.1) [lit.[5] mp 238–240°, $[\alpha]_D$ +416° ($CHCl_3$)]; ir (KBr) 3400 cm^{-1} (OH); NMR δ 2.47, 2.53 (s, 3 H each, 2 NMe), 3.57, 3.75, 3.87 (s, 3 H each, 3 OMe), 5.61 (s, 1 H) (m, 6.21–7.15); mass spectrum *m/e* (rel intensity) 608 (M^+, 38), 607 (24), 501 (6), 471 (1), 417 (4), 381 (59), 192 (100), 191 (78), 176 (10), 175 (15), 168 (19).

***O,O*-Dimethylkrukovine (5).** To a solution of **4** in methanol–ether was added ethereal diazomethane in two portions during 24 hr. The mixture was set aside in the dark. The usual work-up gave **5** as an oil which crystallized from acetone as tiny needles: mp 125°; $[\alpha]_D$ −260° (*c* 0.1); NMR δ 2.33, 2.62 (s, 3 H each, 2 NMe), 3.18, 3.37, 3.74, 3.93 (s, 3 H each, 4 OMe), 6.00 (s, 1 H) (m, 6.30–7.26); mass spectrum *m/e* (rel intensity) 622 (M^+, 100), 621 (40), 515 (<1), 485 (2), 430 (7), 431 (6), 395 (52), 198 (28), 192 (10), 190 (5), 175 (13), 174 (18); high-resolution mass spectrum *m/e* 622.30181 (calcd for $C_{38}H_{42}N_2O_6$, 622.30428).

An authentic sample of phaeanthine, mp 216–217°, was crystallized from acetone to give colorless needles, mp 124–125°, identical in all respects (ir, mixture melting point, TLC) with *O,O*-dimethylkrukovine (**5**).

***O,O*-Bis(trideuteriomethyl)krukovine (6).** To a cooled solution of excess diazomethane in dry dioxane (10 ml) and D_2O (1 ml) was added a solution of **4** (50 mg) in dry dioxane (2 ml) and D_2O (1 ml). After standing for 24 hr in the dark, the usual work-up afforded **6** as an amorphous solid: NMR δ 2.33, 2.61 (s, 3 H each, 2 NMe), 3.37, 3.75 (s, 3 H each, 2 OMe), 6.00 (s, 1 H) (m, 6.29–7.25); mass spectrum *m/e* (rel intensity) 628 (M^+, 100), 627 (77), 626 (35), 521 (<1), 488 (3), 437 (8), 436 (11), 398 (95), 199.5 (68), 192 (22), 190 (6), 175 (35), 174 (37).

Sodium–Ammonia Cleavage of 6. 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 **6** (230 mg) in dry tetrahydrofuran, 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 alowed 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 (100 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 (70 mg).

From the nonphenolic fraction, **9** was isolated by PLC as an oil: NMR δ 2.53 (s, 3 H, 1 NMe), 3.83 (s, 3 H, 1 OMe), 6.10 (s, 1 H),

6.60 (s, 1 H), 6.81 (d, J = 8 Hz, 2 H), 7.06 (d, J = 8 Hz, 2 H); mass spectrum m/e (rel intensity) 333 (M^+ <1), 332 (1), 209 (100), 124 (13).

The oxalate of **9** crystallized from ethanol–ether as colorless needles, mp 127–129°, $[\alpha]_D$ −93° (c 0.14). An authentic sample of (*S*)-*O*,*O*-bis(deuteriomethyl)-*N*-methylcoclaurine was prepared by deuteriomethylation of (*S*)-*N*-methylcoclaurine. Its oxalate crystallized from ethanol–ether as needles, mp 128–129°, $[\alpha]_D$ +101° (c 0.1), and it was found to be identical (ir, mixture melting point, TLC) with the oxalate of **9**.

From the phenolic fraction, **10** was isolated by PLC as an oil: $[\alpha]_D$ −36° (c 0.05); NMR δ 2.42 (s, 3 H, 1 NMe), 3.77 (s, 3 H, 1 OMe), 5.90 (br, 2 H, 2 OH), 6.52 (s, 1 H), 6.29 (s, 1 H), 6.60 (d, J = 8 Hz, 2 H), 6.90 (d, J = 8 Hz, 2 H); mass spectrum m/e (rel intensity) 299 (M^+, 1), 192 (100), 107 (9). The ir ($CHCl_3$) and NMR spectra of **10** were identical with those of an authentic sample of its enantiomer.

A portion of the phenolic fraction (20 mg) was treated with ethereal diazoethane. Work-up in the usual manner after 2 days yielded *O*,*O*′-diethyl-*N*-methylcoclaurine (**11**, 17 mg) as a pale yellow oil. It was converted to the oxalate which crystallized from ethanol–ether as needles: mp 172–174°; $[\alpha]_D$ −114° (c 0.05) (lit.[11] mp 173–174°, $[\alpha]_D$ −123°); mass spectrum m/e (rel intensity) 355 (M^+, 1), 220 (100), 135 (11). The ir spectrum, mixture melting point, optical rotation, and R_f of this compound were identical with those of an authentic sample.

N-Methylation of Daphnoline (3). To a methanol–chloroform solution of daphnoline (**3**, 13 mg), excess of 40% formaldehyde solution was added, and the mixture was stirred at room temperature for 2 hr.

The solution was then cooled in an ice bath and $NaBH_4$ was added in small portions. The solution was further stirred for 1 hr. Work-up as usual gave 13 mg of a transparent oil which crystallized from chloroform as colorless prisms, mp 182–183°, identical (ir, mixture melting point, TLC) with aromoline (**1**).

Acknowledgment. We thank the National Institutes of Health for a grant (CA-11445) in support of this work, and also the Fundación Juan March for the supporting fellowship to one of us (J. M. Saá). We also thank the following individuals for their invaluable assistance: B. A. Krukoff (Honorary Curator of New York Botanical Garden and Consulting Botanist of the Merck Sharp and Dohme Research Laboratories) for the supply of plant material, identification of the voucher sample, and botanical consultation; Drs. I. R. C. Bick (University of Tasmania), M. Tomita (University of Kyoto), and J. R. Boissier (Division Scientifique Roussel Uclaf) for authentic samples of daphnoline, homoaromoline, and phaeanthine, respectively; Drs. C. Hignite and C. Costello (Massachusetts Institute of Technology) for the determination of high-resolution mass spectra.

Registry No. —**1**, 519-53-9; **2**, 17132-74-0; **3**, 479-36-7; **4**, 57377-42-1; **5**, 1263-79-2; **6**, 57288-11-6; **9**, 57256-00-5; **9** oxalate, 57256-01-6; **10**, 5096-70-8; **11**, 6681-71-6.

References and Notes

(1) R. F. Raffauf, "A Handbook of Alkaloids and Alkaloid-Containing Plants", Wiley-Interscience, New York, N.Y., 1970.
(2) B. A. Krukoff and R. C. Barneby, *Mem. N.Y. Bot. Gard.*, **20**, 1 (1970).
(3) B. A. Krukoff and R. C. Barneby, *Mem. N.Y. Bot. Gard.*, **20**, 71 (1970).
(4) I. R. C. Bick, E. S. Ewen, and A. R. Todd, *J. Chem. Soc.*, 2767 (1949).
(5) M. Tomita, M. Kozuka, and M. Satomi, *Yakugaku Zasshi*, **87**, 1012 (1967).
(6) K. J. van der Merwe, P. S. Steyn, and S. H. Eggers, *Tetrahedron Lett.*, 3923 (1965).
(7) I. R. C. Bick, J. Harley-Mason, N. Sheppard, and M. J. Vernengo, *J. Chem. Soc.*, 1896 (1961).
(8) For some general references concerning the mass spectra of bisbenzylisoquinoline alkaloids, see the following: (a) D. C. DeJongh, S. R. Shrader, and M. P. Cava, *J. Am. Chem. Soc.*, **88**, 1052 (1966); (b) M. Tomita, T. Kikuchi, K. Fujitani, A. Kato, H. Furukawa, Y. Aoyogi, M. Kitano, and T. Ibuka, *Tetrahedron Lett.*, 857 (1966); (c) J. Baldas, I. R. C. Bick, T. Ibuka, R. S. Kapil, and Q. N. Porter, *J. Chem. Soc., Perkin Trans. 1*, 592 (1972); (d) J. Baldas, I. R. C. Bick, M. Falco, J. X. deVries, and Q. N. Porter, *ibid.*, 597 (1972); (e) J. Baldas, I. R. C. Bick, T. Ibuka, R. S. Kapil, and Q. N. Porter, *ibid.*, 599 (1972).
(9) A. W. McKenzie and J. R. Price, *Aust. J. Chem.*, **6**, 180 (1953).
(10) M. Shamma, "The Isoquinoline Alkaloids", Academic Press, New York, N.Y., 1972, pp 119–122.
(11) M. Tomita and T. Kikuchi, *Yakugaku Zasshi*, **77**, 997 (1957).
(12) (a) I. R. C. Bick and G. K. Douglas, *Chem. Ind. (London)*, 694 (1965); (b) J. Baldas, I. R. C. Bick, G. K. Douglas, and Q. N. Porter, *Aust. J. Chem.*, **21**, 2305 (1968).

β-Phenethylamine and Tetrahydroisoquinoline Alkaloids from the Mexican Cactus *Dolichothele longimamma*[1]

Richard L. Ranieri and Jerry L. McLaughlin*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

Received July 28, 1975

Seven crystalline alkaloids have been isolated and identified from extracts of a Mexican "peyote" cactus, *Dolichothele longimamma* (DC.) Br. and R. Five of these are new alkaloids: *N*-methyl-β-hydroxy-4-methoxy-β-phenethylamine (longimammine or 4-*O*-methylsynephrine), 6-hydroxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (longimammosine), 8-hydroxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (longimammidine), 6-methoxy-1,2,3,4-tetrahydroisoquinoline (longimammatine), and 4,8-dihydroxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (longimammamine). Although these compounds have all previously been synthesized, a new and convenient route is described for the syntheses of longimammidine and longimammosine. The known cactus alkaloids, (−)-normacromerine and (±)-synephrine, were also found in this species.

The peyote cactus, *Lophophora williamsii* (Lem.) Coult., has a well-documented history as a folkloric medicine and is known to contain many β-phenethylamine and tetrahydroisoquinoline alkaloids including the hallucinogen, mescaline.[2] Several ethnobotanical reports by Schultes suggest that *Dolichothele longimamma* (DC.) Br. and R., another Mexican "peyote" cactus, may cause similar psychoactive effects.[3] Early reports state that this genus contains unknown poisonous alkaloids,[4] and in a recent TLC screen of some *Dolichothele* species several new unidentified cactus alkaloids were detected in *D. longimamma*.[5] In the present communication we report the isolation and structure determination of seven alkaloids from this cactus.

TLC visualization[1a,6] of alkaloid-bearing fractions[5] from freeze-dried and pulverized *D. longimamma* confirmed the presence of several compounds that were distinct from previously known cactus alkaloids.[6,7] Large-scale extraction, involving basification of the plant material, chloroform ma-