STUDIES ON INDONESIAN MEDICINAL PLANTS. V[1]. THE ALKALOIDS OF *ANAMIRTA COCCULUS*

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ABSTRACT.—In the stem and roots of *Anamirta cocculus* the quaternary alkaloids berberine, palmatine, magnoflorine and columbamine were identified. The major tertiary alkaloid was identified as *l*-8-oxotetrahydropalmatine. The structure of this new alkaloid was proven by means of uv, ms, ^{1}H nmr and ^{13}C nmr spectrometry and chemical conversion into *l*-tetrahydropalmatine.

Anamirta cocculus (L.) Wight et Arn. is a liana which occurs in South-East Asia. Berries of this plant are used as fish poison. From the berries the sesquiterpene mixture picrotoxin is commercially isolated (1). The berries have been official in a number of pharmacopoeias. The seed shells of *Anamirta cocculus* were reported to contain alkaloids in 1834 by Pelletier and Couerbe (2). The two alkaloids isolated, menispermine ($C_{18}H_{24}O_2N$) and paramenispermine ($C_{18}H_{24}C_2N$), were probably tertiary because of the isolation procedure followed. No other reports on alkaloids in *Anamirta cocculus* have been published since the work of Pelletier and Couerbe.

RESULTS AND DISCUSSION

In continuation of our studies on Indonesian medicinal plants, we screened the stem and roots of *Anamirta cocculus* for the presence of alkaloids. In a 1% aqueous acetic acid extract, small amounts of alkaloids were found to be present (about 0.1%). From this extract four quaternary alkaloids and one tertiary alkaloid could be isolated. The quaternary alkaloids were identified as berberine, palmatine, magnoflorine and columbamine by means of uv, ^{1}H nmr, mass spectrometry and tlc comparison with reference compounds.

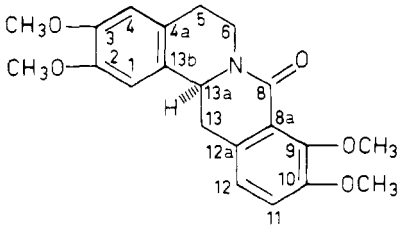
The tertiary alkaloid fraction contained one major component. By means of preparative tlc this alkaloid was obtained pure. The ms showed a molecular weight of 369. In the ^{1}H nmr, signals of four methoxy groups were observed. Furthermore, an AB doublet and a 2-proton singlet were observed in the aromatic region. From these data it was concluded that the alkaloid was a protoberberine derivative, most likely a tetrahydropalmatine derivative. This was further confirmed by means of ^{13}C nmr. In the off-resonance ^{13}C nmr spectrum only three aliphatic triplets were observed and one doublet, indicating a substitution in the 5, 6, 8 or 13 position.

The singlet at 162.6 ppm pointed to the presence of a lactam function, i.e., substitution on C_6 or C_8. The signal of C-5 was observed at 29.4 ppm; this is very similar to the shift of this carbon in tetrahydropseudopalmatine (29.1 ppm) and canadine (29.5 ppm) (3). Therefore, it was concluded that the substituent is in the 8-position. The 4.0 ppm downfield shift of C-11 is also in agreement with an 8-substituent (compared with benzamide where a 2.7 ppm downfield shift is observed for the carbon para to the amide group (4)). The 12.2 ppm upfield

[1]Part IV—see J. Siwon, R. Verpoorte, T. van Beek, H. Meerburg and A. Baerheim Svendsen, *Phytochemistry*, **20**, 323 (1981).

shift of C-6 is similar to the shift observed for the carbons adjacent to the nitrogen in piperine (5), if compared with *N*-methylpiperidine (6) (11.5 ppm upfield). Reduction of the alkaloid with lithium aluminiumhydride yielded *l*-tetrahydropalmatine (yield about 50%), which was purified by means of preparative tlc and identified by means of uv, ms and tlc comparison with a reference compound.

The unknown alkaloid was thus identified as *l*-8-oxotetrahydropalmatine (**1**). This alkaloid has not previously been reported as a natural product. The synthesis of some 8-oxoberbines has been described by Lenz (7). Comparison of the ^{13}C nmr data of 2,3,10,11-tetramethoxy- and 2,3,10,11,12-pentamethoxy-8-oxoberbine (Dr. G. R. Lenz, personal communication) with those of *l*-8-oxotetrahydropalmatine (**1**) showed similar shifts for the A and B ring carbons, C-8 and C-13. Because of different substitution in the D-ring, the other carbons have different shifts.



(**1**) *l*-8-oxotetrahydropalmatine

The occurrence of protoberberine-type alkaloids in *Anamirta cocculus* is not surprising. The genus *Anamirta* belongs to the tribe Cosciniae of the Menispermaceae. The other two genera in this tribe, *Arcangelisia* and *Coscinium*, also contain protoberberine alkaloids (8, 9). Particularly *Arcangelisia* is botanically closely related to *Anamirta*, and *Arcangelisia flava* and *Anamirta cocculus* have sometimes been confused.

The data on the alkaloids menispermine and paramenispermine (2) are insufficient to permit a comparison with the isolated alkaloids described in this paper. Because these alkaloids were isolated from the fruits, we thought it of interest to investigate the fruits for the presence of alkaloids. Only a few berries, Fructus cocculus, from a more than 40-year-old crude drug collection were available. Extraction according to the same method as used for the stem and roots yielded a very small amount of alkaloids. By means of tlc only magnoflorine could be identified clearly. Very faint spots, probably due to berberine, palmatine and *l*-8-oxotetrahydropalmatine, were also observed.

EXPERIMENTAL

Stems and roots of *Anamirta cocculus* (L.) Wight et Arn. (collection number HW1027 (Menispermaceae) were collected by J. Siwon in July 1976 in Pantai Popon (Java, Indonesia). Identification of the plant material was performed by Dr. L. L. Forman, Kew Gardens (London, U.K.). Voucher specimens are kept at the Department of Pharmacognosy (Leiden, The Netherlands).

EXTRACTION METHOD.—Ground stem and roots (1.5 kg) were mixed with 7.5 liters of 1% aqueous acetic acid solution and left for 48 hrs. The extract was filtered off, and the plant material was extracted once more with 4.5 liters of 2% aqueous acetic acid solution for 48 hrs. The combined extracts were concentrated to about ⅓ of the original volume; subsequently,

4 M hydrochloric acid was added until the pH was 1-2. Valser's[2] reagent was added to the solution until no more precipitate was formed.

The precipitate was collected by centrifuging, washed once with distilled water and, subsequently, dissolved in acetone-methanol-water (6+2+1). The solution was passed over a column filled with Amberlite IRA 400 anion exchange resin in the chloride form. The eluate from the column containing the alkaloid chlorides was taken to dryness. The residue was dissolved in water, and 25% ammonia was added until the pH was 9. The basic solution was extracted four times with petroleum ether (40/60) and four times with chloroform. All three fractions were taken to dryness. Yields were: water fraction 1130 mg and chloroform fraction 570 mg, the petroleum ether fraction was a very small amount of only a few milligrams.

COLUMN CHROMATOGRAPHY.—The alkaloids were separated on Merck Lobar ready-made columns filled with Lichroprep Si 60 (63-125 μm) (Size B). As the mobile phase, methanol-water-25% ammonia (8+1+1) was used.

THIN LAYER CHROMATOGRAPHY.—For the identification of the alkaloids the following tlc systems were used:

S1 methanol-water-25% ammonia (8+1+1)
S2 cyclohexane-diethylamine (9+1)
S3 chloroform-methanol-10% ammonia (15+4+1)
S4 ethylacetate-isopropanol-25% ammonia (9+7+1)
S5 chloroform-cyclohexane-diethylamine (10+8+3)
S6 toluene-ethanol-25% ammonia (5+5+1)
S7 *n*-butanol-water-acetic acid (4+1+1)
S8 *n*-hexane-isopropanol (2+1)

All systems were used with Merck Silica gel 60 F_{254} precoated plates in saturated chambers.

Preparative tlc was performed on Silica gel PF_{254}, 1 mm thick plates with the solvent S2 for the separation of berberine and palmatine, and with solvent S8 for the purification of *l*-8-oxotetrahydropalmatine and tetrahydropalmatine. For the elution of the alkaloids from the silica gel, the mobile phase was used.

APPARATUS.—^{1}H nmr spectra were recorded at 100 MHz with a Jeol FT PS 100 apparatus. The spectra of the quaternary alkaloids were recorded in CD_3OD or $CDCl_3$, of the tertiary alkaloid in $CDCl_3$, both with TMS as internal standard. Mass spectra were recorded on an AEI ms 902 at 70 eV, or an LKB 9000 at 12 and 70 eV, both apparatuses were equipped with a direct inlet system. Uv spectra were recorded on a Cary spectrophotometer in methanol solutions. ^{13}C nmr spectra were recorded at 25.15 MHz on a Jeol FT PS 100 apparatus; both proton-noise decoupled and off-resonance spectra were recorded.

Characterization of the alkaloids.

Berberine: The uv spectrum showed maxima at λ: 228, 264 and 364 nm. The mass spectrum of the tetrahydro derivative showed characteristic fragments at (70 eV, 130°) *m/z*: 340(7.5), 339(27)(M^-), 338(13), 324(14), 308(5), 259(24), 241(20), 169.5(2)(M^{2+}), 167(30), 164(33), 149 (100%) and 147(22). ^{1}H nmr showed characteristic signals at ($CDCl_3$, δ in ppm, relative to TMS): 8.229(s,H_{13}), 7.812(s,H_{11},H_{12}), 7.367(s,H_1), 6.839(s,H_4), 6.088(s,methylendioxy protons), 4.309(s,9-OCH_3), 4.059(s,10-O-CH_3), 3.058-2.975(m,H_5,H_6). HRf-values in tlc systems: S1 13, S2 30, S3 39 and S7 47.

Palmatine: The uv spectrum showed maxima at λ: 227, 262-275 and 343 nm. The mass spectrum of the tetrahydro derivative showed characteristic fragments at (70 eV, 200°) *m/z*: 356(26), 355(100%)(M^+), 354(58), 340(10), 324(16), 192(12), 190(28), 177.5(5)(M^{2+}), 165(21), 164(79) and 149(42). The ^{1}H nmr showed characteristic signals at ($CDCl_3$ δ in ppm, relative to TMS): 8.580(s,H_{13}), 8.000, 7.913, 7.840, 7.723 (AB doublets, H_{11},H_{12}), 7.463(s,H_1); 6.799(s,H_4), 4.277(s,9-OCH_3), 4.068(s,10-OCH_3), 4.049(s,2-OCH_3) and 3.973(s,3-OCH_3). HRf-values in tlc systems: S1 13, S2 17, S3 44 and S7 38.

Magnoflorine: The uv spectrum (base) showed maxima at λ: 230, 272(sh), 278 and 325 nm; in acid maxima are observed at λ: 223, 268 and 302 nm. The mass spectrum showed characteristic fragments at (70 eV, 400°) *m/z*: 342(2), 341(7)(M^-), 326(2), 310(1), 298(3), 59(100%) and 58(100%). The ^{1}H nmr (base) showed characteristic signals at (CD_3OD, δ in ppm relative to TMS): 6.505-6.755 (AB doublet, H_9,H_{10}) 6.556(s,H_4), 3.814(s,11-OCH_3), 3.785(s,3-OCH_3), 3.266(s,NCH_3) and 2.896(s,NCH_3). HRf-values in tlc systems: S1 35 and S3 15.

Columbamine: The uv spectrum (base) showed maxima at λ: 238 (sh), 270 and 325 nm; in acid maxima are observed at λ: 227, 264 and 342 nm. The mass spectrum of the tetrahydro derivative showed characteristic fragments at (70 eV, 180°) *m/z*: 342(25), 341(100%)(M^+), 340(60), 326(10), 310(17), 176(16), 165(21), 164(89) and 149(36). The ^{1}H nmr showed characteristic signals at (CD_3OD, δ in ppm, relative to TMS): 9.641(s,H_5), 8.608(s,H_{13}), 7.949(s,H_{11}, H_{12}), 7.548(s,H_1), 6.830(s,H_4), 4.230(s,9-OCH_3), 4.099(s,10-OCH_3) and 3.969(s,3-OCH_3). HRf-values in tlc systems: S1 43 and S3 34.

[2]To prepare Valser's reagent, 10 g of potassium iodide was dissolved in 100 ml of water, added to 14 g of mercury (II) iodide and then filtered.

l-8-oxotetrahydropalmatine: (**1**) The uv spectrum showed maxima at λ: 280, 310, 338(sh) and 365 nm. The mass spectrum showed characteristic fragments at (70 eV, 120°) *m/z*: 370(27), 369(100%)(M^+), 354(18), 340(9), 338(7), 309(14), 274(13), 273(9), 256(10), 251(19), 245(15), 221(44), 220(100), 192(59), 191(27), 184.5(14)(M^{2+}) and 178(100). The ^1H nmr showed characteristic signals at (δ in ppm, relative to TMS): 6.897, 6.973, 6.987, 7.07(AB doublet, H_{11},H_{12}), 6.684(s,H_1,H_4), 4.023(OCH_3), 3.893(3xOCH_3) and 3.001–2.822(m).

The ^{13}C nmr showed signals at (δ in ppm relative to TMS): 162.656(s,C-8), 153.041(s,C-9), 152.26(s,C-10), 147.852(s,C-2,C-3), 130.835(s,C-13b*), 127.617(s,C-4a*), 127.496(s,C-12a*), 123.584(s,C-8a*), 121.977(d,C-12), 115.119(d,C-11), 111.357(d,C-4), 109.052(d,C-1), 61.580 (d/q,9-OCH_3), 56.549(q,2xOCH_3), 55.938(q,OCH_3), 54.996(d,C-13a), 39.223(t,C-6**), 38.161 (t,C-13**) and 29.425(t,C-5). HRf-values in TLC systems: S1 88, S3 90 and S8 27

* and ** interchangeable

Reduction of l-8-oxotetrahydroplamatine: Two mg of *l*-8-oxotetrahydropalmatine were dissolved in 10 ml of anhydrous diethylether, 45 mg $LiAlH_4$ was added, and the mixture was refluxed for 15 hrs. Excess $LiAlH_4$ was destroyed by the addition of ethylacetate. According to tlc, about 50% of the alkaloid was reduced to tetrahydropalmatine. Tetrahydropalmatine was purified by means of preparative tlc.

Tetrahydropalmatine: The uv spectrum showed maxima at λ: 238 and 283 nm. The mass spectrum showed characteristic peaks at (70 eV, 100°) *m/z*: 356(23), 355(100%)(M^+), 354(60), 340(11), 324(18), 192(11), 190(30), 177.5(4)(M^{2+}), 165(25), 164(99) and 149(68). Optical rotation: $[\alpha]^{20}D = -126.6$ (c=0.0047g/100 ml in $CHCl_3$). HRf-values in TLC systems: S1 90, S5 60 and S8 40.

Reduction of berberine, palmatine and columbamine: A small amount of berberine (palmatine or columbamine) was dissolved in methanol and $NaBH_4$ was added until the reduction was completed (followed by tlc). The solution was taken to dryness and then dissolved in basic diethyl ether and filtered. The basic diethyl ether layer was taken to dryness.

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LITERATURE CITED

1. R. Hegnauer, "Chemotaxonomie der Pflanzen", Vol. 5, Birkhäuser Verlag, Basel, Stuttgart, 1969, p. 88.
2. J. Pelletier and P. Couerbe, *Ann.*, **10**, 198 (1834).
3. D. W. Hughes, H. L. Holland and D. B. Maclean, *Can. J. Chem.*, **54**, 2252 (1976).
4. E. Breitmaier and W. Voelter, "^{13}C NMR spectroscopy, Monographs in Modern Chemistry", Vol. 5, 2nd Edition, Verlag Chemie Weinheim, New York, 1978, p. 187.
5. ibid p. 243.
6. M. Shamma and D. M. Hindenlang, "Carbon-13 NMR Shift Assignments of Amines and Alkaloids", Plenum Press, New York and London, 1979, p. 27.
7. G. R. Lenz, *J. Org. Chem.*, **39**, 2846 (1974).
8. C. W. Thornber, *Phytochemistry*, **9**, 157 (1970).
9. J. Siwon, R. Verpoorte, F. A. van Essen and A. Baerheim Svendsen, *Planta Med.*, **38**, 24 (1980).