STUDIES ON INDONESIAN MEDICINAL PLANTS. VII[1]. ALKALOIDS OF *ARCANGELISIA FLAVA*

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Abstract.—From stem and roots of *Arcangelisia flava* six quaternary alkaloids, thalifendine, dehydrocorydalmine, jatrorrhizine, pycnarrhine, berberine and palmatine and three tertiary alkaloids, hydroxy-berberine, limacine and homoaromoline, have been isolated and identified by their spectral data.

The genus *Arcangelisia* belongs, together with the genera *Coscinium* and *Anamirta*, to the tribe Coscieae of the Menispermaceae. The genus *Arcangelisia* comprises two species (Forman, 1). *A. flava* (L.) Merr is a liana found in South-East Asia. *A. tympanopoda* (Lauberb. & K. Schum.) Diels is a liana found so far only in New-Guinea. The two species are differentiated by the size of the fruits, the latter one having larger fruits than the former.

Various medicinal uses of *A. flava* have been reported: as a febrifuge, tonic, abortive, expectorant and emmanogogue and against hepatitis and indigestion (Santos (2), van Steenis (3), Kondo (4), Estrada *et al.* (5), Quisumbing (6)). Wells (7) reported the presence of berberine in the stem of *A. flava*. Santos (2) identified berberine, jatrorrhizine and columbamine and isolated an alkaloid which was called "shobakunine". This was later shown to be a mixture of palmatine and berberine (8). Garcia *et al.* (9) detected berberine, palmatine and jatrorrhizine in the stem and berberine and jatrorrhizine in the roots of *A. loureirii* Diels (a synonym for *A. flava* (1)).

RESULTS AND DISCUSSION

By means of column chromatography and preparative tlc a series of alkaloids was isolated from the roots and stems of *A. flava*. The alkaloids were identified by means of their spectral data and tlc comparison with reference substances previously isolated in our laboratories. The major alkaloids were the quaternary protoberberine alkaloids berberine and jatrorrhizine and the simple isoquinoline alkaloid pycnarrhine (10). As minor alkaloids, dehydrocorydalmine (11) and thalifendine (12) were found as well as palmatine in minute amounts. 8-Hydroxy-berberine, which was isolated from a chloroform fraction, may be an artifact due to the isolation method employed. Furthermore, the tertiary bisbenzyl-isoquinoline alkaloids limacine and homoaromoline were identified (10).

Berberine, palmatine and jatrorrhizine had previously been detected in *A. flava* by Santos (2) and Garcia *et al.* (9). However, columbamine, which was reported by Santos (2), could not be detected in our plant material.

EXPERIMENTAL

Plant material.—The plant material was collected by Harry Wiriadinata in August 1976 under field number HW 1039 near Pantai Ngliyep, south of Malang on Java, Indonesia. The plant was identified as *Arcangelisia flava* (L.) Merr. by Dr. L. L. Forman of Royal Botanic Gardens, Kew, England. A voucher specimen is kept in our laboratories.

Extraction.—Stem and roots (485 g) were ground and extracted twice with 3 liters of 3% aqueous acetic acid. The extracts were concentrated under reduced pressure, and the pH was adjusted to pH = 1–2 with 1% hydrochloric acid. The precipitate formed was collected, dissolved in dilute hydrochloric acid, and processed separately as the filtrate. To the filtrate 0.1 M Mayer's reagent was added until no more precipitate was formed. The precipitate was collected by means of centrifuging and was subsequently dissolved in acetone-methanol-water (6:2:1). The solution was passed through Amberlite IRA 400 anion exchange resin in chloride

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form, yielding the alkaloid chlorides. The solution, when taken to dryness, yielded 8.2 g of crude alkaloid chlorides. The alkaloid chlorides were dissolved in water and 25% ammonia was added to make the pH about 9. The basified solution was extracted twice with diethyl ether and twice with chloroform. The diethyl ether and the chloroform extracts, when evaporated to dryness, yielded, respectively, 40 mg and 200 mg of tertiary alkaloids. Evaporation of the aqueous phase produced about 8 g of quaternary alkaloids. The precipitate formed after acidification of the original extract yielded, after the procedure described above, 6 mg of alkaloids extracted with diethyl ether, 35 mg of alkaloids extracted with chloroform, and 600 mg was isolated from the aqueous phase. The total amount of alkaloids isolated from the plant material corresponds to an alkaloid content of about 2%.

SEPARATION OF THE ALKALOIDS.—The quaternary alkaloids were separated by means of column chromatography. Merck Silica gel 60 ready made columns (size A for the aqueous fraction, size B for further purification of the alkaloids) were used in combination with the solvent methanol-water-25% ammonia (8:1:1).

Thalifendine and dehydrocorydalmine were obtained pure by means of recycling chromatography on two coupled Merck Silica gel 60 ready made columns size A with toluene-ethanol-25% ammonia (5:5:1) as the mobile phase. Pycnarrhine was isolated by chromatography on a Merck Silica gel 60 column size A by use of the mobile phase chloroform-methanol-10% ammonia (14:5:1). The two tertiary alkaloids from the diethyl ether extract were obtained by column chromatography on a Merck Silica gel 60 column size A and toluene saturated with 25% ammonia-absolute ethanol (4:1) as mobile phase. As the final purification step, preparative tlc on 1 mm thick silica gel plates in combination with solvent system S7 was applied.

TLC.—For the identification of the alkaloids the following solvent systems were used:

S1 methanol-water-25% ammonia (8:1:1)
S2 toluene-96% ethanol-25% ammonia (5:1:1)
S3 cyclohexane-diethylamine (9:1)
S4 chloroform-methanol-10% ammonia (14:5:1)
S5 *n*-butanol-water-acetic acid (4:1:1)
S6 ethyl acetate-isopropanol-25% ammonia (8:8:5)
S7 ethyl acetate-isopropanol-25% ammonia (9:7:1)
S8 cyclohexane-chloroform-diethylamine (10:8:3)

All mobile phase were used in combination with Merck Silica gel 60 ready made plates in saturated chromatography chambers. The alkaloids were detected in uv light of 254 nm and 366 nm and by spraying with iodoplatinate spray reagent.

Thalifendine.—The uv spectrum (in methanol) showed maxima at 224, 275, 338–350 and 390 nm under neutral conditions, 226, 265, 276 and 352 nm under acidic conditions and 290, and 378 nm under basic conditions. Ms of the tetrahydro compound showed characteristic fragments *m/z* (70 eV, 100°) 326(9), 325(45) (M^+), 324(27), 294(4), 207(27), 206(24), 205(21), 191(24), 177(27), 176(100), 175(30), 174(24), 162.5(2) (M^{2+}), 151(30), 150(91), 135(42), 112(45), 111(42), 109(39) and 107(36).

The ^{1}Hnmr spectrum showed signals at (CD_3OD, 100 MHz, δ in ppm relative to TMS) 9.41(s, H–8), 8.51(s, H–13), 7.84, 7.75, 7.70, 7.60 (AB-d, H–11 and H–12), 7.59(s, H–1), 6.92 (s, H–4), 6.07(s, O–CH_2–O) and 4.08(s, 9–OCH_3).

Dehydrocorydalmine.—The uv spectrum (in methanol) showed maxima at 224, 275, 338–350 and 390 nm under neutral conditions, 226, 265, 276 and 352 nm under acidic conditions and 290 and 378 nm under basic conditions. Ms of the tetrahydro compound showed characteristic fragments *m/z* (70 eV, 150°) 342(14), 341(52) (M^+), 340(32), 326(37), 310(13), 205(15), 193(27), 192(27), 192(100), 191(16), 190(32), 170.5(3) (M^{2+}), 151(35), 150(87), 135(68), 111(58), 109(69) and 107(34).

The ^{1}Hnmr spectrum showed signals at (CD_3OD, 100 MHz, δ in ppm relative to TMS) 9.51(s, H–8), 8.67(s, H–13), 7.92, 7.81, 7.75, 7.64 (AB-d, H–11 and H–12), 7.63(s, H–1), 7.04(s, H–4), 4.12(s, 9–OCH_3), 3.98(s, 2–OCH_3) and 3.93(s, 3–OCH_3).

Jatrorrhizine.—The uv spectrum (in methanol) showed maxima at 228, 240, 262, 350 and 430 nm under acidic conditions and 248 and 396 nm under basic conditions. Ms of the tetrahydro compound showed characteristic fragments *m/z* (70 eV, 140°) 342(21), 341(100) (M^+), 340(50), 326(5), 310(12), 176(17), 170.5(4) (M^{2+}), 165(17) and 164(67).

The ^{1}Hnmr spectrum showed characteristic signals at ($CDCl_3$ to which some CD_3OD is added, 100 MHz, δ in ppm relative to TMS) 9.80(s, H–8), 8.57(s, H–13), 7.94 (broad s, H–11 and H–12), 7.48(s, H–1), 6.91(s, H–4), 4.25(s, 9–OCH_3), 4.10(s, 10–OCH_3) and 4.05(s, 2–OCH_3).

Pycnarrhine.—The uv spectrum (in methanol) showed maxima at 252, 313 and 370 nm under acidic conditions and 250, 283(s), 304 and 335 nm under basic conditions. Ms showed characteristic fragments *m/z* (70 eV, 140°) 194(9), 193(69), 192(77) (M^+), 191 (metastable peak 193→192), 177(15), 163 (metastable peak 192→177), 151(12), 150(100), 135(12) and 121.5 (metastable peak 150→135).

The ^{1}Hnmr spectrum showed signals at (CD_3OD, 100 HMz, δ in ppm relative to TMS), 8.77(s, H–1), 7.17(s), 7.07(s) (H–5 and H–8), 4.04(s, OCH_3), 4.01(t, H–3), 3.70(s, NCH_3) and 3.25(t, H–4).

The ^{13}Cnmr spectrum showed signals at (CD_3OD, 25.15 MHz, δ in ppm relative to TMS), 166.41(C–1), 157.81(C–6), 147.69(C–7), 131.94(C–4a), 119.84(C–8a), 118.60(C–8), 112.17(C–5), 57.13(OCH_3), 51.55(C–3), 46.42(NCH_3) and 25.96(C–4).

Berberine.—The uv spectrum (in methanol) showed maxima at 230, 266, 340(s), 351 and 428 nm under acidic conditions and 255, 288 and 362 nm under basic conditions. Ms of the tetrahydro compound showed characteristic fragments *m/z* (70 eV, 180°) 340(25), 339(100) (M^+), 338(53), 324(8), 308(6), 169.5(1) (M^{2+}) and 164.

Palmatine.—The uv spectrum (in methanol) showed maxima at 268 and 339 nm under acidic conditions and 275 and 350 nm under basic conditions.

Hydroxy-berberine.—The uv spectrum (in methanol) showed maxima at 259 and 350 nm under acidic conditions and 262–275 and 348 nm under neutral and weakly basic conditions. Ms showed characteristic fragments *m/z* 70 eV, 300°) 338(35), 337(100) (M^+–0), 320(11), 306(10), 278(13), 256(10), 202(33), 201(16), 200(25), 199(19), 190(11), 187(13), 186(11), 176(15), 173(23), 168.5(9), 159(43), 149(23), 145(30) and 144(55).

The ^{1}Hnmr spectrum showed signals at ($CDCl_3$, 100 MHz, δ in ppm relative to TMS), 7.16(s, H-13), 7.02, 6.95, 6.90, 6.82 (AB-d, H-11 and H-12), 6.62(s, H-1), 6.10(s, H-4), 5.95 (s, OCH_2–O), 5.64(s, H-8), 3.94(s, 9-OCH_3) and 3.84(s, 10-OCH_3).

Limacine.—The uv spectrum (in methanol) showed a maximum at 282 nm. Ms showed characteristic fragments *m/z* (70 eV, 270°) 609(13), 608(31) (M^+), 607(16), 593(4), 471(1), 381(21), 368(3), 367(11), 347(38), 198(18), 193(88), 192(100), 191(50), 177(29), 174(11) and 168(11).

The ^{1}Hnmr spectrum showed characteristic signals at ($CDCl_3$, 100 MHz, δ in ppm relative to TMS), 7.49–6.05 (aromatic protons), 3.93(s, 12-OCH_3), 3.79(s, 6-OCH_3), 3.39(s, 6'-OCH_3), 2.70(s, 2'-NCH_3) and 2.41(s, 2-NCH_3). $[\alpha]^{20}D = -190.5°$ (c=0.003 g/100 ml in methanol).

Homoaromoline.—The uv spectrum (in methanol) showed a maximum at 282 nm. Ms showed characteristic fragments *m/z* (70 eV, 300°), 609(5), 608(10) (M^+), 607(5), 537(16), 478(9), 462(15), 452(26), 408(17), 391(22), 382(4), 381(8), 367(6), 365(9), 198(100), 191(35), 175(31) and 167(35).

The ^{1}Hnmr spectrum showed characteristic signals at ($CDCl_3$, 100 MHz, δ in ppm relative to TMS), 6.95–6.38 (aromatic protons), 3.90(s, 12-OCH_3), 3.85(s, 6'-OCH_3), 3.61(6-OCH_3), 2.70(2-NCH_3) and 2.64(s, 2'-NCH_3). $[\alpha]^{20}D = +153.4°$ (c=0.010 g/100 ml in methanol).

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