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ALKALOIDAL AND OTHER CONSTITUENTS OF *UNCARIA ELLIPTICA* AND *CANTHIUM DICOCCUM**

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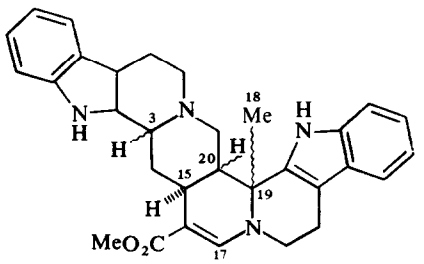
Key Word Index—*Uncaria elliptica*; *Canthium dicoccum*; Rubiaceae; roxburghines D and X; formosanine; mitraphylline; sitosterol; quinovaic acid; acetylquinovaic acid; scopoletin.

Abstract—A new alkaloid roxburghine X, along with roxburghine D, formosanine and mitraphylline, has been isolated from the bark of *Uncaria elliptica*. Sitosterol, quinovaic acid, acetylquinovaic acid and scopoletin were isolated from the bark of *Canthium dicoccum*.

INTRODUCTION

Uncaria elliptica (R.B. ex G.Dm.) and *Canthium dicoccum* (Gaertn.) Merr belong to the family Rubiaceae and are endemic to Sri Lanka. Both plants grow in the wet lowland forests of the island. *U. elliptica* is a woody climber while *C. dicoccum* is a moderately sized tree. In an earlier paper [1], we reported the isolation and identification of 3 new ursene carboxylic acids from the woody part of *U. elliptica*. In this paper we report the isolation of the alkaloids roxburghine D, roxburghine X, formosanine and mitraphylline from the bark of the same plant. Roxburghine X is a hitherto unknown stereoisomer of roxburghine D, earlier isolated from an *Uncaria* species [2].



Roxburghine X

The bark, timber and leaves of *C. dicoccum* contained only trace quantities of alkaloids but sitosterol, quinovaic acid, acetylquinovaic acid and scopoletin were isolated from the bark. Quinovaic acid is an ursene carboxylic acid previously isolated from other Rubiaceae [3–5].

RESULTS AND DISCUSSION

The alkaloidal fraction of the bark of *U. elliptica* was isolated by moistening with ammonia and macerating with ethyl acetate [6]. Chromatography over alumina gave roxburghine X, formosanine, mitraphylline and roxburghine D. The fractions eluted from the column were monitored by TLC using various spray reagents which distinguished between the different structural types [7].

High resolution MS showed that roxburghine X had the molecular formula $C_{31}H_{32}O_2N_4$. The molecular formula and reaction with ceric sulphate suggested a roxburghine type of alkaloid. The MS fragmentation pattern as well as the ^{1}H NMR, UV and IR data confirmed the roxburghine type. However, the mp and specific rotation of roxburghine X were not in agreement with those reported for roxburghines A, B, C, D, or E [2].

Assuming the absolute configuration at C-15 to be α, as in almost all the loganin-derived indole alkaloids, there are 8 possible roxburghines. Four of these have *trans*-fused D/E rings, i.e. H(15α), H(20β), while four have *cis*-fused D/E rings, i.e. H(15α), H(20α). Roxburghines C, D and E have been shown to have the absolute configurations H(3α), H(15α), H(20β), C(18α); H(3β), H(15α), H(20β), C(18α); and H(3β), H(15α), H(20β), C(18β), respectively, whilst roxburghine B has the absolute configuration H(3β), H(15α), H(20α), C(18β) [8, 9].

Dehydrogenation of the roxburghines B, C, D and E with iodine and sodium acetate leads to aromatization of ring D and resulting loss of chirality at C-3, C-15 and C-20 [2]. Roxburghines C and D give the same dehydroroxburghine, since they have the same configuration at C-19, the chiral centre not affected in the dehydrogenation. Attempted dehydrogenation of roxburghine X with iodine and sodium acetate, however, gave a complex mixture. Hence all the data available to us indicate that roxburghine X either has *trans*-fused D/E rings and is the 3α-epimer of roxburghine E, or that it has *cis*-fused D/E rings like roxburghine B. With the data available to us at the moment, it is not possible to decide about the fusion of rings D and E.

Unnamed alkaloids have been isolated from several species of *Canthium* [10, 11], while the peptide alkaloid canthiumine has been isolated from *C. euryoidesis* [12]. In the present work only traces of alkaloids were found in the bark, timber and leaves of *C. dicoccum*.

* Part 34 in the series "Chemical Investigation of Ceylonese Plants". For Part 33 see ref. [1].

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The bark of *C. dicoccum* was extracted with hot petrol and sitosterol separated out from the extract. The residual bark was extracted with hot acetone and the extract taken up in ether. The ether extract, on standing, gave a solid which was separated by PLC into quinovaic acid and acetylquinovaic acid. Evaporation of the ether, followed by chromatography of the residue over silica gel gave scopoletin.

EXPERIMENTAL

U. elliptica and *C. dicoccum* were collected in January at the Udawattakelle Forest Reserve near Kandy, Sri Lanka. ^{1}H NMR spectra were recorded at 60 MHz with TMS as internal reference. Mps were taken on a Kofler block and are uncorr. Petrol had bp 60–80°.

Isolation of roxburghine X, formosanine, mitraphylline and roxburghine D from U. elliptica. Dry powdered bark (275 g) was moistened with 10% NH_4OH and macerated with EtOAc. The conc EtOAc extract was shaken with 2% H_2SO_4, made alkaline with NH_4OH, the bases extracted with $CHCl_3$, the $CHCl_3$ extract washed with H_2O, dried (Na_2SO_4) and evapd. A brown solid (4.03 g) was obtained. This solid (4 g) was chromatographed over Al_2O_3 (200 g).

Elution of the column with C_6H_6–$CHCl_3$ (20:1) gave a light pink solid which on PLC ($CHCl_3$) gave roxburghine X as a faint pink crystalline solid (30 mg), mp 215° (from EtOH); $[\alpha]_D^{27}$ −29° (MeOH). (Found: M^+, 492.253. $C_{31}H_{32}O_2N_4$ requires: M^+, 492.599); MS *m/e* (%): 492 (52) (M^+), 491 (7), 477 (5), 461 (4), 433 (7), 362 (3), 336 (3), 331 (5), 321 (4), 307 (7), 306, 294, 293, 279, 269, 247, 235, 222, 221 (100), 211, 208, 198, 197, 184 (10), 183, 182, 171 (5), 169, 156 (8), 144 (7), 130 (4), 129; IR ν_{max}^{Nujol} cm^{-1}: 3415, 3320, 1680, 1620, 1470, 1390, 1250, 1225, 1170, 1132, 1090, 1020, 940, 745; UV λ_{max}^{EtOH} nm: 226 (log ε 4.91), 284 (4.60), 292 (4.58); ^{1}H NMR ($CDCl_3$): δ 8.5 (1H, *s*, N-H), 8.26 (1H, *s*, N-H), 7.4 (1H, *s*, C_{17}-H), 7.36–7.1 (8H, *m*, aromatic H), 4.3 (1H, *m*, C_3-H), 3.52 (3H, *s*, —$COOCH_3$), 3.2–2.7 (14H, *m*, $6CH_2$ + 2CH), 1.58 (3H, *s*, C_{19}-CH_3).

Elution of the column with C_6H_6–$CHCl_3$ (9:1) gave a white solid which on PLC ($CHCl_3$) gave white crystals of formosanine (40 mg), mp 129° (from EtOH); $[\alpha]_D^{27}$ +100.2° (EtOH) (lit. [13] mp 130°, $[\alpha]_D$ +106.5°). Mmp and TLC with authentic specimen established identity. MS *m/e* (%): 368 (25) (M^+), 351, 337, 223 (71), 208, 149 (12), 146 (4), 145 (6), 144 (7), 130 (8), 69 (100); IR ν_{max}^{Nujol} cm^{-1}: 3307, 2840, 1707, 1617, 1302, 1277, 1187, 1097, 757; UV λ_{max}^{EtOH} nm: 210 (log ε 4.18), 243 (4.02), 285 (2.9); ^{1}H NMR ($CDCl_3$): δ 7.8 (1H, *s*, N_1—H), 7.32 (1H, *s*, C_{17}—H), 7.16–6.73 (4H, *m*, aromatic H), 4.38–4.2 (2H, *m*, C_3—H and C_{19}—H), 3.56 (3H, *s*, $COOCH_3$), 2.51–2.0 (10H, *m*, $4CH_2$ + 2CH), 1.11 (3H, *d*, C_{19}—CH_3).

Elution of the column with C_6H_6–$CHCl_3$ (3:1) gave a white solid which on PLC ($CHCl_3$) gave white needles of mitraphylline (2 g), mp 275° (from EtOH); $[\alpha]_D^{27}$ −4.2 ($CHCl_3$) (lit. [13] mp 276°, $[\alpha]_D^{24}$ −3°). Mmp and TLC with authentic sample established identity. MS *m/e* (%): 368 (41) (M^+) 353, 351, 337, 224, 223 (100), 208, 194, 191, 180, 162, 144, 136, 130, 122, 108, 94; IR ν_{max}^{Nujol} cm^{-1}: 3189, 2840, 1728, 1698, 1615, 1447, 1381, 1294, 1227, 1189, 1176, 1126, 1090, 924, 759; UV λ_{max}^{EtOH} nm: 210 (log ε 4.32), 243 (4.11), 287 (2.3); ^{1}H NMR ($CDCl_3$): δ 8.33 (1H, *s*, N_1—H), 7.2 (1H, *s*, C_{17}—H), 7.2–6.96 (4H, *m*, aromatic H), 4.63–4.3 (2H, *m*, C_3—H and C_{19}—H), 3.6 (3H, *s*, $COOCH_3$), 2.53–2.06 (10H, *m*, $4CH_2$ + 2CH), 1.13 (3H, *d*, C_{19}—CH_3). Identity was further confirmed by isomerization with hot Py [14] to isomitraphylline, mp 306°.

Elution of the column with C_6H_6–$CHCl_3$ (1:1) gave a pale yellow solid which on PLC ($CHCl_3$–MeOH, 9:1) gave faint yellow crystals of roxburghine D, mp 200° (from EtOH): $[\alpha]_D^{27}$ +150° (MeOH) (lit. [2] mp 197–200°, $[\alpha]_D^{20}$ +160° (MeOH). MS *m/e* (%): 492 (15) (M^+), 491 (4), 477 (11), 461 (2), 434, 368, 362 (2), 353, 336 (4), 321 (6), 307 (7), 305, 293, 279, 265, 247, 234, 223, 221, 208, 197, 184 (100), 171 (16), 156 (30), 144 (19), 130 (19); IR ν_{max}^{KBr} cm^{-1}: 3410, 2920, 1670, 1620, 1450, 1390, 1310, 1240, 1100, 1065, 1020, 930, 750; UV λ_{max}^{EtOH} nm: 224 (log ε 4.89), 284 (4.61), 290 (4.57); ^{1}H NMR ($CDCl_3$): δ 8.16 (1H, *s*, N—H), 7.33 (1H, *s*, C_{17}—H), 7.26–6.83 (8H, *m*, aromatic H), 4.5 (1H, *m*, C_3—H), 3.66 (3H, *s*, —$COOCH_3$), 3.4–2.7 (14H, *m*, $6CH_2$ + 2CH), 1.46 (3H, *s*, C_{19}—CH_3). Reaction with I_2 and NaOAc [2] gave dehydroroxburghine D, mp >300°. Reaction with Pb tetraacetate followed by reaction with $NaBH_4$ [8] gave roxburghine C, mp 245°; mmp and TLC identical with an authentic sample.

The yields of roxburghine X, formosanine, mitraphylline and roxburghine D per dry wt of bark were 0.003, 0.009, 0.19 and 0.002%, respectively.

Isolation of sitosterol, quinovaic acid, acetylquinovaic acid and scopoletin from C. dicoccum. Dried powdered bark (800 g) was extracted with hot petrol. On standing, a white solid separated from the extract. Recrystallization gave sitosterol (2.7 mg), mp 136° (from petrol), $[\alpha]_D^{26}$ −35° ($CHCl_3$) (lit. [13] mp 137°, $[\alpha]_D$ −36° ($CHCl_3$)). Mmp and TLC with authentic sample established identity. The residual bark was extracted with hot Me_2CO. Evapn of solvent gave a brown solid, which was taken up in Et_2O. On standing, the Et_2O extract gave a white solid (100 mg) which on PLC ($CHCl_3$–MeOH, 99:1) gave quinovaic acid (60 mg) and acetylquinovaic acid (10 mg). Quinovaic acid had mp 297° (from EtOH), $[\alpha]_D^{27}$ +85° (KOH) (lit. [5] mp 298°, $[\alpha]_D$ +87° (KOH)). Mmp and TLC with authentic sample established identity. IR ν_{max}^{KBr} cm^{-1}: 3400, 2910, 2830, 1670, 1450, 1380, 1250, 1050, 950, 760; acetylquinovaic acid had mp 280° (from MeOH) (lit. [5], mp 281°). Mmp and TLC with authentic sample, prepared by acetylation of quiovaic acid established identity. MS *m/e* (%): 528(1) (M^+) 484, 469, 409, 379, 278, 273, 261, 249, 233, 227, 215, 206, 205, 203, 191, 190(100), 189, 175, 163, 161, 147, 135, 133, 121, 119, 109, 107, 105; IR ν_{max}^{KBr} cm^{-1}: 2970, 1730, 1710, 1460, 1390, 1245, 1150.

Evapn of the mother liquor of the Et_2O extract gave a brown solid (1.00 g) which after chromatography on Si gel (70 g) gave faint yellow needles of scopoletin (300 mg), mp 203° (from *n*-hexane) (lit. [13] mp 204°). Mmp and TLC with authentic specimen established identity. The yields of sitosterol, quinovaic acid, acetylquinovaic acid and scopoletin per dry wt of bark were 0.34, 0.08, 0.04 and 0.04%, respectively.

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