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THREE NEW URSENE CARBOXYLIC ACIDS FROM *UNCARIA THWAITESII**

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Key Word Index—*Uncaria thwaitesii*; Rubiaceae; ursene carboxylic acids; sitosterol; ursolic acid.

Abstract—Three new ursene carboxylic acids, uncaric acid, diketouncaric acid and diacetyluncaric acid were isolated from the woody part of *Uncaria thwaitesii*, interrelated and their structures established.

INTRODUCTION

Indole alkaloids of several ring types have been isolated from species of *Uncaria* [2–12]. However there is no information about the presence of terpenoid compounds in the genus. In the present communication, we report the determination of the structures of three new ursene carboxylic acids, uncaric acid, diketouncaric acid and diacetyluncaric acid isolated from *Uncaria thwaitesii* (Rubiaceae). The plant is a woody climber which grows in the wet lowland forests of Sri Lanka. It is endemic to Sri Lanka and is the only species of *Uncaria* to be found in the island.

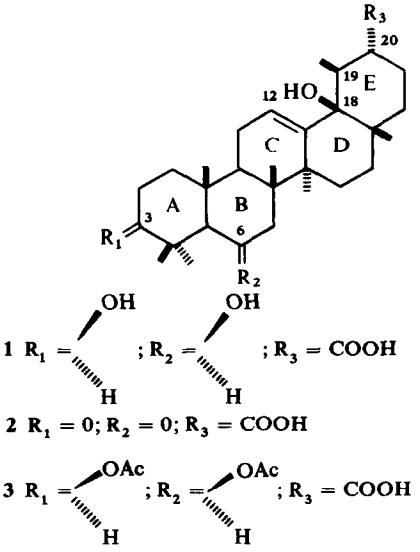
RESULTS AND DISCUSSION

The woody part of the plant was extracted with hot petrol. Sitosterol and ursolic acid were isolated from this extract, after removal of trace amounts of alkaloids. The residual plant material was extracted with hot acetone, the extract taken up in ether, alkaloidal matter eliminated and the residue chromatographed on Si gel. Uncaric acid (**1**), diketouncaric acid (**2**) and diacetyluncaric acid (**3**), were obtained in reverse order of elution from the column.

MS indicated that **1**, **2** and **3** had molecular weights of 488, 484, and 572, respectively. High resolution MS indicated that **1** had the molecular formula $C_{30}H_{48}O_5$. The IR spectrum showed absorption for hydroxyl (3363 cm^{-1}), for carboxyl (1698 cm^{-1}) and for a trisubstituted double bond (853 cm^{-1}). The NMR spectrum had signals for C<u>H</u>(OH) at δ 4.60 (1H, *m*) and 3.25 (1H, *m*); for C<u>H</u>(COOH) at 2.40 (1H, *m*) and for an olefinic proton at 5.50 (1H, *m*). Signals for methyl groups were observed in the region δ 1.35–1.03 (21H).

Oxidation of **1** with chromium trioxide in pyridine gave two compounds, the less polar of which was shown to be identical with **2**. High resolution MS showed that the more polar compound **4** had the molecular formula $C_{30}H_{46}O_5$. The NMR spectrum of **4** showed a signal for C<u>H</u>OH at δ 3.15 (1H, *m*) while the NMR spectrum of **2** showed no such signal, indicating that **4** was a monoketouncaric acid while **2** was a diketouncaric acid. Compound **2** could not be further oxidized and its IR spectrum showed absorption for hydroxyl, indicating the presence of two secondary hydroxyl groups and possibly a tertiary hydroxyl group in **1**. The NMR spectrum of **2** showed well resolved signals for six tertiary methyl groups in the region δ 1.49–0.80 and one secondary methyl group at 0.94 (3H, *d*, J = 6 Hz) showing that uncaric acid (**1**) is an ursene containing a carboxyl group in place of one of the secondary methyl groups.

Acetylation of **1** with pyridine and acetic anhydride gave two compounds, the major product being identical with **3**. High resolution MS indicated that **3** had the molecular formula $C_{34}H_{52}O_7$. Further comparison of IR and NMR spectra of **1** and **3** showed that **3** is a diacetyluncaric acid. The minor product of acetylation, **5**, was more polar than **3**. The MS of **5** showed a molecular weight of 530, indicating that **5** was a monoacetyluncaric acid. Compound **3** showed IR absorption for hydroxyl but no NMR signals for C<u>H</u>(OH). Compound **3** could not be further acetylated giving further evidence for the presence of two secondary hydroxyl groups and one tertiary hydroxyl group in **1**.



1 R_1 = ; R_2 = ; R_3 = COOH

2 R_1 = 0; R_2 = 0; R_3 = COOH

3 R_1 = ; R_2 = ; R_3 = COOH

* Part 33 in the series "Chemical Investigation of Ceylonese Plants". For Part 32 see ref. [1].

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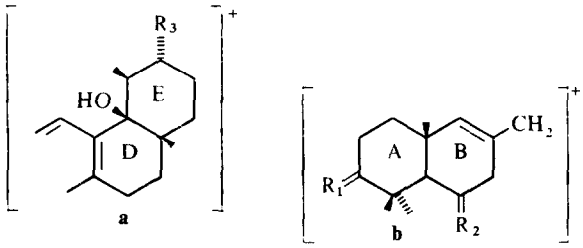
The MS of 1, 2 and 3 gave definite evidence for the presence of a Δ^{12}-ursene skeleton. The characteristic fragmentation in such compounds [13, 14] is the collapse of ring C in a retro Diels–Alder reaction to give ions **a** and **b**.



In uncaric acid (1), significant peaks were observed at *m/e* 264 and 223 for **a** (R_3 = COOH) and **b** (R_1 = R_2 = H, OH). In the methyl ester (6) of (1), ion **a** appeared at *m/e* 278, showing the presence of the carboxyl group of 1 in ring E. The MS of 2 and 3 showed ion **a** at *m/e* 264, while ion **b** appeared in 2 at *m/e* 219 and in 3 at *m/e* 307, showing that the two secondary hydroxyl groups in 1 are in the A,B ring system while the tertiary hydroxyl group is in the D,E ring system. MS provided further evidence for the presence of a tertiary hydroxyl group in the D,E ring system. Significant peaks for the loss of water from ion **a** were observed in the MS of 1, 2, 3 and 6.

In an ursene skeleton, a tertiary hydroxyl group in the D,E ring system could be at C-18, C-19 or C-20. Since both the secondary methyl group and the secondary carboxyl group are in ring E, the tertiary hydroxyl group in uncaric acid (1) and its derivatives can only be at C-18. The carboxyl group should be at C-20 rather than at C-19 since the NMR spectra of 1, 2 and 3 showed a multiplet for C<u>H</u>(COOH) rather than a doublet.

One of the secondary hydroxyl groups in 1 was placed at C-3 on biogenetic grounds. The β-configuration of the hydroxyl group was evident from the large $W_{1/2}$ values (17–20 Hz) [15] for the axial C-3 proton in the NMR spectra of 1, 3 and 4.

The shift [16] in the 23β-methyl signal from δ 1.03 in the NMR spectra of 1 to 0.95 in the monoacetate (5) provided confirmation for the 3β-hydroxyl group in 1 and also indicated a 3β-acetoxyl group in 5.

The other secondary hydroxyl group in uncaric acid (1) could be neither at C-2 nor at C-1, since the UV spectrum of the diketo compound (2) resembled neither the UV spectrum of cyclohexan-1,2-dione nor the UV spectrum of cyclohexan-1,3-dione. Hence this group in 1 could only be at either C-6 or C-7.

The NMR spectra of both the monoketo compound (4) and the diketo compound (2) showed singlets at δ 2.48 which were assigned to the C-5 proton. This indicated that the secondary hydroxyl groups in uncaric acid (1) were C-3 and C-6 and that 4 was 6-ketouncaric acid. Further, the NMR spectra of uncaric acid (1), as well as that of its diacetyl derivative (3), showed multiplets for the equatorial C-6 proton ($W_{1/2}$ = 6 Hz). The low $W_{1/2}$ values indicated 6β-hydroxyl in 1 and 6β-acetoxyl in 3 [15].

Hence uncaric acid (1) was 3β,6β,18β-trihydroxyurs-12-en-oic-acid, diketouncaric acid (2) was 18β-hydroxy-3,6-dioxours-12-en-30-oic acid and diacetyluncaric acid (3) was 3β,6β-diacetoxy-18β-hydroxurs-12-en-30-oic acid.

EXPERIMENTAL

Uncaria thwaitesii (Hook. f.) Alston was collected in January at Udawattakelle forest reserve near Kandy, Sri Lanka. Mps were taken on a Kofler block and are uncorr. Identity of compounds was established by mp, mmp, TLC and IR.

Isolation of 1, 2 *and* 3. The dried woody part of *Uncaria thwaitesii* (3.25 kg) was extracted with hot petrol (bp 60–80°) for a few days. Evapn of the solvent gave a brownish yellow solid (6.5 g), which was dissolved in Et_2O, washed with dil. HCl, then with H_2O, dried (Na_2SO_4) and the Et_2O evapd. A brown oily solid (5.56 g) was obtained. Si gel chromatography of a portion (3 g) of the solid gave sitosterol (1.71 g) mp 136° and ursolic acid (0.09 g) mp 290°. A portion of the residual plant material (2.25 kg) was extracted with hot Me_2CO for 5 days. Evapn of the solvent gave a reddish brown semi solid, which was taken up in Et_2O, washed with H_2O, then with dil. HCl again with H_2O, dried (Na_2SO_4) and the Et_2O evapd, when a brown solid (2.5 g) was obtained. A portion (2.2 g) of the brown solid was chromatographed in petrol on Si gel (90 g). Elution of the column with petrol–C_6H_6 (1:1) yielded 3 (10 mg), mp 220° (from EtOH), $[\alpha]_D^{27}$ + 73° ($CHCl_3$) (Found: M^+, 572.369. $C_{34}H_{52}O_7$ requires: M^+, 572.755); MS: *m/e* (rel. int.) 572(1) (M^+), 554, 526, 510, 452, 437, 307(5), 264(10), 246(23), 219, 201, 188, 187, 146(100); IR ν_{max}^{KBr} cm^{-1}: 3360, 2870, 1723, 1670, 1453, 1369, 1248, 1140, 851, 810, 730, 650; NMR (100 MHz, $CDCl_3$): δ 5.56 (1H, *m*, $W_{1/2}$ = 6 Hz; C-6), 5.37 (1H, *m*, C-12), 4.44 (1H, *m*, $W_{1/2}$ = 20 Hz, C-3), 2.5 (1H, *m*, C-20), 2.04 (3H, *s*, OCOC<u>H</u>$_3$), 2.00 (3H, *s*, OCOC<u>H</u>$_3$), 1.22–0.92 (21H).

Elution of the column with C_6H_6 gave 2 (15 mg), mp 265° (from EtOH), $[\alpha]_D^{27}$ + 82° ($CHCl_3$); MS *m/e* (rel. int.): 484(1) (M^+), 466, 438, 405, 366, 354, 264(5), 246(38), 233, 219(46), 201, 187, 146(100); IR ν_{max}^{Nujol} cm^{-1}: 3600, 2869, 1709, 1690, 1449, 1379, 1239, 1150, 670; UV λ_{max}^{EtOH} nm: 208(log ε 4.45), $\lambda_{max}^{EtOH-OH^-}$ nm: 210(log ε 5.01), $\lambda_{max}^{EtOH-H^+}$ nm; 215(log ε 3.98); NMR 100 MHz, $CDCl_3$): δ 5.48 (1H, *m*, C-12), 2.57 (1H, *m*, C-20), 2.48 (1H, *s*, C-5), 2.16 (4H, *m*, C-2 and C-7), 1.49 (3H, *s*, C<u>H</u>$_3$), 1.27 (3H, *s*, C<u>H</u>$_3$), 1.24 (6H, *s*, C<u>H</u>$_3$), 1.1 (3H, *s*, C<u>H</u>$_3$), 0.94 (3H, *d*, *J* = 6 Hz, C<u>H</u>$_3$), 0.8 (3H, *s*, C<u>H</u>$_3$).

Elution of the column with $CHCl_3$–MeOH (99:1) gave 1 (120 mg) mp 270° (from EtOH–H_2O), $[\alpha]_D^{27}$ +65° (EtOH) (Found: M^+, 488.348. $C_{30}H_{48}O_5$ requires: M^+, 488.689); MS *m/e* (rel. int.): 488(4), 470, 452, 442, 437, 424, 409, 391, 370, 264(31), 246(67), 231, 223(10), 219, 218, 206, 201(100), 187, 180, 173, 147, 146, 133, 131: IR ν_{max}^{KBr} cm^{-1}: 3363, 2863, 1698, 1453, 1373, 1238, 1153, 1023, 943, 923, 853, 810, 763, 663; NMR (60 MHz, $CDCl_3$, + 10% C_5D_5N): δ 5.5 (1H, *m*, C-12), 4.6 (1H, *m*, $W_{1/2}$ = 6 Hz, C-6), 3.25 (1H, *m*, $W_{1/2}$ = 20 Hz, C-3), 2.4 (1H, *m*, C-20). 2.05–1.76 (18H, C<u>H</u>$_2$), 1.35–1.03 (21H, *m*, C<u>H</u>$_3$)

The yields of 1, 2 and 3 per dry wt of plant material were 0.007, 0.0009 and 0.0007%, respectively, while the yields of sitosterol and ursolic acid were 0.097 and 0.005%, respectively.

Methylation of 1 *to* 6. A soln of 1 (20 mg) in MeOH (5 ml) was treated with an excess of CH_2N_2. Evapn of the solvent gave 6 (12 mg), mp 138–140° (from MeOH), $[\alpha]_D^{27}$ + 6.3° ($CHCl_3$); MS *m/e* (rel. int.):502(3) (M^+), 484, 466, 451, 442, 425, 424, 409, 391, 370, 278(11), 260(25), 250, 245, 223(5), 219, 206, 201, 187, 180(100), 146, 123, 119; IR ν_{max}^{Nujol} cm^{-1}: 3409, 2869, 1719, 1629, 1449, 1379, 1239, 1213, 1199, 1150, 1079, 1019, 939, 919, 769.

Oxidation of 1 *to* 2 *and* 4. CrO_3 (40 mg) was dissolved in H_2O (0.1 ml) and gradually added to Py (3 ml) cooled to 15°. 1 (40 mg) in Py (3 ml) was then added. The mixture was kept for 15 min and poured into H_2O at room temp, extracted with Et_2O, the Et_2O extract washed with H_2O dried (Na_2SO_4) and the Et_2O evapd to give a white solid (35 mg). PLC (Si gel, $CHCl_3$) gave 2 white solids. One of them (15 mg) mp 265° was identical with 2. The other was 4 (17 mg) mp 260° (from EtOH), $[\alpha]_D^{27}$ + 93° ($CHCl_3$) (Found: M^+, 486.334. $C_{30}H_{46}O_5$ requires: M^+, 486.673); MS *m/e* (rel. int.): 486(4) (M^+), 440, 422, 407, 389, 368, 350, 264(13), 246(28), 235, 231, 221(7), 219, 218, 203, 201, 187, 185, 173, 147, 146(100), 144; IR ν_{max}^{KBr} cm^{-1}: 3500, 2870, 1700,

1685, 1450, NMR (240 MHz, $CDCl_3$) 1379, 1239, 1160, 679; NMR 240 MHz, $CDCl_3$): δ 5.4 (1H, *m*, C-12), 3.15 (1H, *m*, $W_{1/2}$ = 20 Hz, C-3), 2.55 (1H, *m*, C-20), 2.48 (1H, *s*, C-5), 2.35 (2H, *m*, C-2) 1.35–0.75 (21H, $C\underline{H}_3$).

Acetylation of **1** *to* **3** *and* **5**. **1** (30 mg) was treated with Py–Ac_2O (1:1) (3 ml) at room temp. for 24 hr and the reaction product poured into ice cold H_2O, acidified with 5 N HCl (4 ml) and extracted with Et_2O. The Et_2O extract was washed with H_2O, dried (Na_2SO_4) and the Et_2O evapd, to give a white solid (28 mg) PLC (Si gel, C_6H_6) gave 2 white solids, one of them (17 mg) mp 220° was identical with **3**. The other was **5** (7 mg) mp 301–303° (from MeOH), $[\alpha]_D^{27}$ + 61.2° ($CHCl_3$); MS *m/e* (rel. int.):530(3) (M^+), 512, 484, 470, 524, 437, 412, 391, 267(17), 264(34), 246(59), 231, 219, 218, 206, 201(100), 187, 173, 147, 119; IR ν_{max}^{KBr} cm^{-1}: 3463, 2863, 1720, 1650, 1453, 1360, 1242, 1160, 923, 840, 810, 764, 660; NMR (60 MHz, $CDCl_3$): δ 5.36 (1H, *m*, C-12), 4.60–4.53 (2H, *m*, C-3 and C-6), 2.55 (1H, *m*, C-20), 2.05 (3H, *s*, $OCOC\underline{H}_3$), 1.68–1.54 (18H, $C\underline{H}_2$), 1.31–0.95 (21H, $C\underline{H}_3$).

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