Pergamon

0031-9422(95)00428-9

Phytochemistry, Vol. 40, No. 4, pp. 1311-1312, 1995
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0031-9422/95 $9.50 + 0.00

URSENE CARBOXYLIC ACIDS OF *UNCARIA ELLIPTICA*

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(*Received* 2 *February* 1995)

Key Word Index—*Uncaria elliptica*; Rubiaceae; triterpenes; uncaric acid.

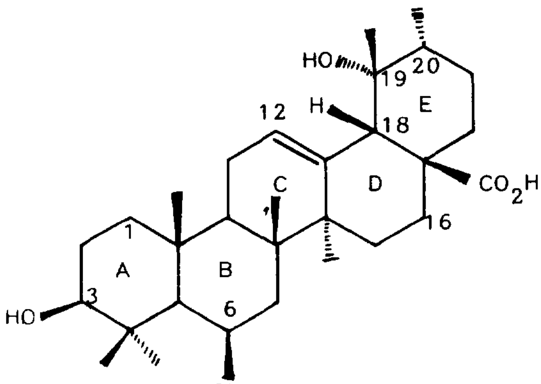
Abstract—The structures of three triterpenoid acids isolated from *Uncaria elliptica* have been revised following a spectroscopic analysis of the major acid, uncaric acid.

INTRODUCTION

Uncaria elliptica R. Br. *ex* G. Don is a woody climber of the family Rubiaceae, growing mainly in the wet lowland forests of Sri Lanka. It is the only species of *Uncaria* to be found in the island. Plants described as *U. thwaitesii* are now classified as *U. elliptica*. In an earlier investigation, the plant, collected in the lower montane zone near Kandy and described as *U. thwaitesii*, was examined for terpenoids. Three ursene carboxylic acids, named uncaric acid, diketouncaric acid and diacetyluncaric acid, were isolated [1]. Their structures were established by spectroscopic methods as well as chemical interrelation. The alkaloids of the same sample of *U. elliptica* have also been investigated [2].

The present investigation was undertaken primarily because the attachment of the carboxyl group to C-20 in the three ursene carboxylic acids appeared biogenetically unusual. Moreover, alternative structures with a $CH_3CH(CO_2H)$ group attached to a five membered E-ring could not be excluded from the available spectroscopic data.

19α-Hydroxylated ursenes have been isolated from *U. florida* [3] and *U. tomentosa* [4]. Common to both species is an acid, identified as 3β,6β,19α-trihydroxyurs-12-en-28-oic acid (**1**). On the basis of ^1H and ^{13}C NMR spectroscopy, particularly connectivities obtained from two-dimensional ^1H-^1H and ^1H-^{13}C correlation methods, the structure of uncaric acid has been revised as **1**.



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RESULTS AND DISCUSSION

The plant material for the present investigation was collected in the wet lowland forest zone. The woody part was extracted successively with hot petrol and acetone, and the alkaloidal matter was removed from each extract by an acid wash. Chromatography of the petrol extract on silica gel gave sitosterol in 0.016% yield along with traces of diketouncaric acid and diacetyluncaric acid. The identity of these acids was established by TLC comparison with specimens obtained by oxidation and acetylation, respectively, of uncaric acid. Traces of ursolic acid were probably present in the petrol extract, but its presence could not be established on TLC evidence alone. Chromatography of the acetone extract on silica gel gave sitosterol and uncaric acid in yields of 0.001 and 0.003%, respectively. Neither diketouncaric acid nor diacetyluncaric acid were present in the acetone extract. It is noteworthy that the triterpenoid composition of the same *Uncaria* species collected at two sites differed considerably, indicating that these plants were probably ecotypes/varieties of the same species.

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Table 1. ^{13}C and ^{1}H NMR data for compound **1** in $CDCl_3$–pyridine-d_5 (9 : 1)*†

C	^{13}C	^{1}H (J_{HH} Hz)
1	40.2	1.56 *m*, 1.00 *m*
2	26.9	1.75 *m*, 1.63 *m*
3	78.1	3.19 *dd* (11.3, 4.5)
4	39.2	
5	55.3	0.77 *d* (1.9)
6	67.4	4.57 ($W_{1/2}$ = 8)
7	40.4	1.75 *m*, 1.56 *m*
8	38.5	
9	47.1	1.71 *m*
10	35.9	
11	23.0	2.11 *m*, 2.04 *m*
12	128.0	5.44 *br t* (3.6)
13	137.5	
14	41.3	
15	27.9	2.0 *m*, 1.1 *m*
16	25.2	2.63 *ddd* (13.1, 13.1, 4.7), 1.68 *m*
17	47.1	
18	53.1	2.71 *s*
19	72.2	
20	40.9	1.43 *m*
21	25.7	1.81 *m*, 1.77 *m*
22	37.2	1.87 *m*, 1.77 *m*
23	27.4	1.11 *s*
24	16.6	1.28 *s*
25	16.1	1.34 *s*
26	17.1	1.18 *s*
27	23.8	1.36 *s*
28	180.2	
29	26.5	1.25 *s*
30	15.7	0.96 *d* (6.8)

**s* = singlet; *d* = doublet; *t* = triplet; *m* = multiplet; *br* = broad.

†Assignments confirmed by attached proton test (APT), ^{1}H–^{1}H double quantum filtered correlation spectroscopy (DQCOSY) and ^{1}H–^{13}C pulsed field gradient indirect detection experiments (GHMQC, GHMBC), using standard pulse sequences.

The spectroscopic data as well as the reactions described earlier [1] were consistent with structure **1** for uncaric acid in rings A, B and C, with a tertiary hydroxyl group in the D, E-ring systems. The singlet at δ2.71 in the ^{1}H NMR spectrum was identified as H-18, indicating that C-17 and C-19 were fully substituted. The carboxyl group and the tertiary hydroxyl group should then be attached to C-17 and C-19, respectively, in the E-ring of the ursene. The equatorial proton at C-16 appearing at δ1.68 showed four-bond coupling with H-18. The axial proton at C-16, which appeared as a triplet of doublets, was shifted downfield to δ2.63 due to the axial hydroxyl group at C-19. A five membered ring structure with an attached CH_3CH (CO_2H) group for uncaric acid was excluded since H-18 was a singlet. Further, the carboxyl carbon showed no correlation with any methine protons except H-18.

Neither diketouncaric acid nor diacetyluncaric acid have been isolated from *U. florida* and *U. tomentosa*. From the established relationship of the two acids to uncaric acid, their structures should be revised to 3,6-dioxo-19α-hydroxyurs-12-en-28-oic acid (**2**) and 3β,6β-diacetoxy-19α-hydroxyurs-12-en-28-oic acid (**3**), respectively.

Uncaric acid showed no antifungal activity (*Cladosporium cladosporioides*) and no larvicidal activity (*Aedes albopictus*).

EXPERIMENTAL

General. Mps: uncorr; ^{1}H, ^{13}C NMR at 500/125 MHz; chemical shifts (δ) in ppm, referenced to $CDCl_3$ (^{1}H, 7.25 ppm; ^{13}C, 77.0 ppm); IR, $[\alpha]_D$ and MS [1].

Plant material. Uncaria elliptica was collected in July from the Kanneliya forest reserve and identified by one of us (A.W.).

Extraction and sepn. The dry ground woody part of *U. elliptica* (2.0 kg) was sequentially extracted with hot petrol (bp 60–80°) and hot Me_2CO. Evapn of the petrol gave a yellowish green semi-solid (3.0 g). Evapn of the Me_2CO gave a reddish brown semi-solid (3.2 g). The petrol extract was taken up in Et_2O, washed with dil. HCl, H_2O, dried (Na_2SO_4) and the Et_2O evapd giving a brown oily solid (2.7 g). This solid was chromatographed on silica gel (30 g) and eluted with *n*-hexane–EtOAc, when sitosterol (324 mg), mp 136° was obtained from the *n*-hexane–EtOAc (12:1) fr. Compounds **2** and **3** were detected by TLC in this fraction. The Me_2CO extract was also taken up in Et_2O and subjected to acid wash, as for the petrol extract, and a brown solid (1.8 g) obtained. This solid was chromatographed on silica gel (75 g) and eluted with petrol, C_6H_6, CH_2Cl_2 and MeOH. Further purification of the benzene eluate by prep. TLC on silica gel gave sitosterol (12 mg). The benzene–CH_2Cl_2 (1 : 1) eluate was purified by prep. TLC over silica gel to give **1** (60 mg), mp 268–270° ($EtOH$–H_2O), identical with available specimen (Co-TLC, IR). For ^{13}C and ^{1}H NMR: see Table 1.

Acknowledgements—The authors thank the Natural Resources, Energy and Science Authority of Sri Lanka for a research grant. They are also grateful to Prof. Y. Fujimoto, of the Tokyo Institute of Technology, for the larvicidal testing.

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