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A PENTACYCLIC TRITERPENE ACID, WITH ANTI-ULCER PROPERTIES, FROM *CUSSONIA NATALENSIS**

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Key Word Index—*Cussonia natalensis*; Araliaceae; pentacyclic triterpene acid, ^{1}H and ^{13}C NMR.

Abstract—The structure of 23-hydroxy-3-oxo-urs-12-en-28-oic acid, isolated from the twigs and leaves of *Cussonia natalensis*, has been determined by spectrometric methods

INTRODUCTION

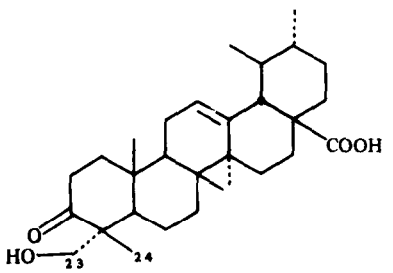
Cussonia natalensis Sonder is a small tree occurring in Natal and the Northern and Eastern parts of Transvaal [1]. *Cussonia* is used in folk medicine for a variety of complaints such as pain, inflammation and infection [2].This paper deals with the isolation and structure elucidation of a new triterpene acid [**1**] from *C. natalensis*, for which anti-ulcer properties have been found. Oleanolic acid was also isolated from this plant.

RESULTS AND DISCUSSION

Compound **1**, $C_{30}H_{46}O_4$, showed in its IR spectrum (Nujol) typical bands for an hydroxy (3472 cm^{-1}) and a carbonyl group (1690 cm^{-1}). It also indicated the presence of an acid (3210 cm^{-1} – 2300 cm^{-1}). Hydrogen bonding between the 23-hydroxy and 3-oxo groups resulted in overlap of the absorption of the latter with that of the acid carbonyl at 1690 cm^{-1}. This hydrogen bonding is consistent with a chair form for ring A [3].

The ^{1}H NMR spectrum of **1** showed four tertiary methyl groups at δ0.82, 0.98, 1.10 and 1.12 (positions C-24, C-25, C-26, C-27) and two secondary methyl groups at δ0.83 and 0.93 (positions C-29 and C-30, J = 6.4 Hz). These features, together with the presence of one olefinic proton at δ5.23 (H-12), confirmed the ursane framework of **1** [4]. The ^{1}H chemical shifts of the hydroxy-methyl AB protons (δ3.40 and 3.62, J = 11.3 Hz) were in close agreement with those of methyl 23-hydroxy-3-oxo-olean-12-en-28-oate (δ3.42 and 3.65, J = 11.2 Hz) and different from those of methyl 24-hydroxy-3-oxo-urs-12-en-28-oate (δ3.46 and 3.95 J = 11.1 Hz) [4], indicating a C-23 rather than a C-24 hydroxy group for **1**.

Likewise, the ^{13}C NMR chemical shifts assigned to the A ring carbon atoms of **1** correlated well with those of the methyl 23-hydroxy-3-oxo-oleane compound but were different from those of the methyl 24-hydroxy derivative [4]. Specific differences of 1.3 and 6.7 ppm were observed in the shifts of C-4 and C-5 respectively. The C-24 resonance of the 23-hydroxy compound was observed 4.8 ppm upfield from that of the C-23 resonance of the 24-hydroxy derivative and the corresponding hydroxymethyl group carbons had a 1 3 ppm difference in shift, again confirming a C-23 position for the hydroxy group in **1**.



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EXPERIMENTAL

Plant material The twigs and leaves of *C natalensis* were collected by Prof. A. E Van Wyk during December 1984, 33 km east of Pietersburg, South Africa. A voucher specimen (AvW NO. 6908) is deposited in the Schweickerdt Herbarium at the University of Pretoria.

Extraction and isolation. Air-dried plant material (4 kg) was extracted with C_6H_6, EtOAc and MeOH and worked-up in the usual fashion [5]. Compound **1** was isolated from a fraction eluted from a silica gel column (silica gel 60) with EtOAc–petrol (4:1).

23-Hydroxy-3-oxo-urs-12-en-28-oic acid (**1**). Colourless crystals from EtOAc (0.02% yield): mp 170°, IR ν_{max}^{nujol} cm^{-1}: 3472 (OH), 1690 (C=O), 1044 (CH_2OH); MS *m/z* (rel. int.): 470.34 [M]$^+$ (2) (calc for $C_{30}H_{46}O_4$ 470.36), 440 (2), 424 (2), 248 (40), 203 (30), 133 (63), 105 (25), 91 (25), 44 (100); ^{1}H NMR (500 MHz $CDCl_3$): δ0.82 (3H, *s*, Me), 0.83 (3H, *d*, J = 6.4 Hz, Me), 0.93 (3H, *d*, J = 6.2 Hz, Me), 0 98 (3H, *s*, Me), 1.10 (1H, *s*, Me), 1.12 (1H, *s*, Me), 3 40 (1H, *d*, J = 11.3 Hz, CH_2OH), 3 62 (1H, *d*, J = 11 3 Hz, CH_2OH), 5.23 (1H, *b*, *s*, J = 3 Hz, CH), ^{13}C NMR (75 MHz $CDCl_3$). δ15.3 (*q*, C-25), 16.8 (*q*, C-26), 17 0 (*q*, C-29), 17.2 (*q*, C-24), 19 1 (*t*, C-6), 21.1 (*q*, C-30), 23.4 (*t*, C-11), 23.6 (*q*, C-27), 24.0 (*t*, C-16), 28.0 (*t*, C-15), 30.6 (*t*, C-21), 32.3 (*t*, C-7), 35.2 (*t*, C-2), 36.5 (*t*, C-22), 36.7 (*s*, C-10), 38.8 (*d*, C-20), 38.8 (*t*, C-1), 39.0 (*d*, C-19), 39 5 (*s*, C-8), 42.1 (*s*, C-14), 46.6 (*d*, C-9), 48 0 (*s*, C-17), 49.0 (*d*, C-5), 52 4 (*s*, C-4), 52.6 (*d*, C-18), 67.0 (*t*, C-23), 125 4 (*d*, C-12), 138.2 (*s*, C-13), 183.6 (*s*, C-28), 219 0 (*s*, C-3).

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SPECTROSCOPIC DETERMINATION OF STRUCTURES OF TRITERPENOID TRISACCHARIDES FROM *CENTELLA ASIATICA*

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Key Word Index—*Centella asiatica*, Umbelliferae, terminolic acid, asiaticoside-A, asiaticoside-B, triterpenoid saponin

Abstract—The structures of two new triterpenoid trisaccharides asiaticoside-A and asiaticoside-B from *Centella asiatica* have been elucidated as the [*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl(1→6)]-*O*-β-D-glucopyranose ester of 2α,3β,6β,23α-tetrahydroxy-urs-12-ene-28-oic acid and the [*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl (1→6)]-*O*-β-D-glucopyranose ester of 2α,3β,6β,23α-tetrahydroxyolean-12-ene-28-oic acid by spectroscopic analysis

INTRODUCTION

In a previous communication [1] the molecular geometry of asiaticoside, the major triterpenoid trisaccharide from *Centella asiatica* has been reported. The widespread reputation of the plant in India and Madagascar for the treatment of leprosy [2, 3] prompted us to investigate the plant for the isolation and characterization of other constituents of biological interest. It may be mentioned that considerable phytochemical studies [4–9] have already been done on this plant species

RESULTS AND DISCUSSION

Usual solvent extraction of the air-dried leaves and repeated CC purification of the ethanolic extract led to the isolation of two glycoside fractions. The fraction of lower polarity could be crystallized and was characterized as asiaticoside [1]. The fraction of slightly higher polarity designated CA-2 although apparently homogeneous by TLC, turned out to be a mixture of two components as revealed by HPLC and ^{13}C NMR. Successful separation and isolation of the pure components by HPLC was frustrated by their similar polarity. However, negative FAB-mass spectrometry of CA-2 with thioglycerol as matrix exhibited ion peaks at *m/z* 973 and 1009 ascribable to $[M-H]^-$ and $[M-H+2H_2O]^-$, respectively. Positive FAB mass spectrometry with diethanolamine (DEA) as matrix showed the ion peak at *m/z* 1080 attributable to $[M+DEAH]^+$. On the other hand positive FAB mass spectrometry of CA-2 with thioglycerol as matrix showed the highest mass ion peak at *m/z* 997 assignable to $[M+Na]^+$. Thus, the FAB mass spectra of CA-2 suggested that the M_r of both of its components are the same which is 974. The ^{13}C NMR spectrum of CA-2 disclosed that both of its constituents have the sugar moiety attached to the carboxyl groups of their aglycones which possess the same M_r but different skeletons. Moreover, the ^{13}C NMR spectrum revealed that the carbohydrate moiety of CA-2 is identical to that of asiaticoside [1] whose ^{13}C chemical shifts have completely been assigned. Hydrolysis of CA-2 afforded a mixture of two aglycones which could be separated by HPLC on a S-10-ODS reversed-phase column using acetonitrile–water (3:2) as the mobile phase. The two aglycones were characterized by mass and ^{13}C NMR spectral analysis as 6β-hydroxy asiatic acid (**1**) [6] and terminolic acid (**2**), the latter being isolated for the first time from a natural source other than *Terminalia arjuna* [10]. The acid **1** belongs to the ursane series and the acid **2** to the oleanane group. Assignments of the ^{13}C chemical shifts of the acids **1** and **2** were straightforward using known chemical shift rules [11], off-resonance studies and by comparison of their shift data with those of triterpenes with similar skeletons [12,13] especially asiatic acid [1] and arjunolic acid [14], respectively, taking into consideration the 6β-hydroxylation effect [15]. Noting the glycosylation shift values [16,17], comparison of the ^{13}C data of the acids **1** and **2** as well as their common carbohydrate moiety with those of CA-2 led to the assignments of the ^{13}C chemical