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Phytochemistry, Vol 28, No 10, pp 2852–2854, 1989
Printed in Great Britain

0031 9422/89 $3.00 + 0.00

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

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(*Received* 5 *December* 1988)

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

shifts of the two glycosides designated as asiaticoside-A (3) and asiaticoside-B (4) (Table 1) constituting CA-2. We are pursuing our studies to evaluate the antileprotic activity of CA-2.

EXPERIMENTAL

Mps uncorr. IR spectra were recorded in Nujol mulls. ^{1}H NMR in $CDCl_3$ at 99.6 MHz and ^{13}C NMR at 25.05 MHz for C_5D_5N solns with TMS as int std MS were obtained at 70 eV HPLC was performed on a Spherisorb S-10-ODS reversed-phase column (25 cm × i.d. 10 mm) using a RI detector.

Extraction and isolation of glycosides. Air-dried *C asiatica* (1 kg) was successively extracted with petrol (60–80°), $CHCl_3$ and 90% EtOH The extract on removal of solvent under red. pres. yielded a viscous dark brown mass This was extracted with *n*-BuOH and the extract washed with H_2O to remove H_2O sol inorganic impurities and free sugars, then evapd to dryness under red pres to give a dark brown residue (45 g). The residue was chromatographed over silica gel (600 g) and eluted with petrol, petrol–$CHCl_3$ (1 1), $CHCl_3$, $CHCl_3$–MeOH (9.1, 17:3, 41.9, 4.1) A total of 105 frs (250 ml each) was collected and frs having the same R_f values on TLC were combined Frs eluted with $CHCl_3$–MeOH (41 9) on repeated chromatographic purification followed by crystallization from MeOH furnished needles of asiaticoside (1.8 g), mp 230–232° (decomp), $[\alpha]_D$ −16°(MeOH; *c* 0.52). Elution of the column with $CHCl_3$–MeOH (4:1) yielded a solid (1.2 g) which on further purification by chromatography, afforded a TLC homogeneous amorphous glycoside fraction CA-2, mp 200–205° (decomp).

Hydrolysis of CA-2 and isolation of 6β-hydroxy asiatic acid (1) *and terminolic acid* (2). Fr. CA-2 was a mixt of two compounds by HPLC. However, isolation of pure compounds by prep. HPLC was not successful. Fr. CA-2 (1.9 g) was hydrolysed with 2M HCl in aq. MeOH (75 ml) at 100° for 4 hr. Usual work-up yielded a mixt of two aglycones (720 mg). Prep. HPLC of genin mixt on a Spherisorb-S-10-ODS reversed-phase column with MeCN–H_2O (3 2) and flow rate 2.2 ml/min afforded 6β-hydroxy asiatic acid (360 mg) and terminolic acid (230 mg).

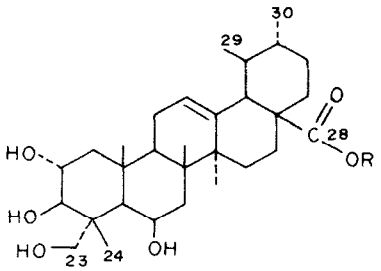
6β-Hydroxy asiatic acid (1). Crystallized from MeOH as a white powder (350 mg), mp 285–288°; Me ester, mp 168–170°; $[\alpha]_D$ +30° ($CHCl_3$, *c* 0.82), triacetate Me ester, mp 134–136° $[\alpha]_D$ −8.5° ($CHCl_3$, *c* 1.9) [lit. [6], Me ester, mp 167–169°, $[\alpha]_D$ +34° (EtOH), 6β-hydroxy-2α,3β,23-triacetate Me ester, mp 142–145°, $[\alpha]_D$ −5.9° (EtOH)].

Terminolic acid (2). Crystallized from aq MeOH as colourless powder (200 mg) mp >300° $[\alpha]_D$ +44.2° (EtOH; *c* 0.28); Me terminolate (MeOH) mp 165–168°, $[\alpha]_D$ +37° ($CHCl_3$; *c* 0.6); [lit. [10], Me ester, 165–168°, $[\alpha]_D$ +40° ($CHCl_3$; *c* 0.78)]. HRMS

Table 1. ^{13}C chemical shifts (± 0.1) of compounds **1–3** in pyridine-d_5

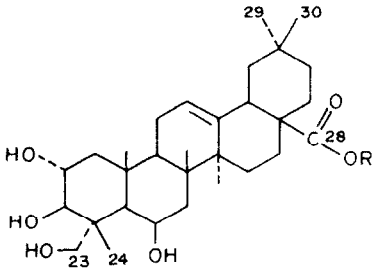
C	1	2	3	4	C	3	4
1	48 2	46 9	48.2	47.0	glc-2	73.6	73.6
2	69 0	68.8	68.9	68 9	glc-3	78.3	78.3
3	78 5	78 3	78.7	78 5	glc-4	70.1	70.4
4	43 1	43.1	43.1	42.8	glc-5	76.7	76.7
5	48 6[a]	48 1	48.7	48.7	glc-6	71.0	71.0
6	67 6	67.6	67 6	67 9	glc-1′	104.4	104.0
7	39.0	39 2	39 3	39.3	glc-2′	75 0	75.0
8	39 5	40.0	39 5	39.5	glc-3′	77.6	77.6
9	48 9	48 9	49.7	49 5	glc-4′	78.3	78.3
10	38 0	38.4	37 9	38.0	glc-5′	76.3	76.3
11	25 0[b]	23.8[a]	24.3	23 9	glc-6′	61.4	61.6
12	126.0	122.5	126.3	122.5	rha-1	102.4	102.4
13	138 6	144 0	137 7	143.4	rha-2	72.4[a]	72 4[a]
14	44 3	42.2	44 2	44 2	rha-3	72.1[a]	72.1[a]
15	28 7	28.1	28 7	28.0	rha-4	73 6	73.6
16	26 0[b]	23 3[a]	24 5	23 9	rha-5	69.4	69.4
17	48 0[a]	43.0	48 4	43 1	rha-6	18.1	18.1
18	53 3	41.9	53.3	42.8			
19	38 1	46 1	38 0	46.6			
20	39.1	30 5	39.0	30 6			
21	31 0	33.8	31 0	33.0			
22	37 5	32.5	37.5	32.5			
23	66 6	66.6	66 5	66 5			
24	15 5	15.3	15 5	15 5			
25	17 2	17 5	17.4	17.4			
26	18.6	18 5	19 1	19 1			
27	23 8	23.7	23 7	24 3			
28	179 8	179 3	176.2	176.4			
29	24.0	33 1	23.7	33 0			
30	21.3	23.9	21 1	24 3			
glc-1			95.5	95 5			

glc = Glucose, rha = rhamnose. [a,b]Signals may be interchanged in each vertical column.



1 R = H

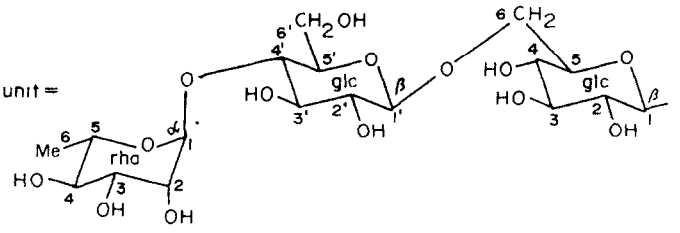
3 R = trisaccharide unit



2 R = H

4 R = trisaccharide unit

Trisaccharide unit =



m/z (rel intensity) 518 3600 $[M]^+$ (Calcd for $C_{31}H_{50}O_6$, 518 3607) (1 1), 500 3483 $[M-H_2O]^+$ (0.9), 482.3380 $[M-2H_2O]^+$ (2.7), 459.3453 $[M-COOMe]^+$ (0 97), 458 3373 $[M-COOMe-H]^+$ (1), 423 3253 ($C_{29}H_{43}O_2$) (1 9), 302.2248 ($C_{20}H_{30}O_2$) (2 1), 263 1970 ($C_{17}H_{27}O_2$) (16 5), 262 1936 ($C_{17}H_{26}O_2$) (79), 204 1839 ($C_{15}H_{24}$) (16 2), 203 1805 ($C_{15}H_{23}$) (100), 202 1727 ($C_{15}H_{22}$) (16 1) and 189 1652 ($C_{14}H_{21}$) (18 1) 2α,3β,23-Triacetoxy-6β-hydroxy-methyl terminolate as leaflets (MeOH), mp 164–165°, $[\alpha]_D+15^c$ ($CHCl_3$, *c* 1 1), [lit [10], methyl-tri-*O*-acetylterminolate, mp 160–162° $[\alpha]_D+19°$ ($CHCl_3$, *c* 1.53)] ^1H NMR ($CDCl_3$) δ 0 76 (3H, *s*), 0 86 (3H, *s*), 0 89 (3H, *s*), 0 98 (3H, *s*), 1.2 (3H, *s*), 1 26 (3H, *s*) (6 × Me), 1.96 (3H, *s*, OAc), 2 02 (3H, *s*, OAc), 2 06 (3H, *s*, OAc), 3 6 (3H, *s*, COOMe), 3.68 and 3 82 (2H, ABq, $-CH_2OAc$, $J=12$ Hz), 4 36 (1H, *br s*, –OH), 4 98 (*d*, $J=8$ Hz, H-3), 5 12 (*m*, H-2) and 5 32 (*t*-like, 1H, H-12)

Acknowledgements—Our sincere thanks are due to Dr B N Pramanik (Schering Corporation, Bloomfield, New Jersey, U S A) for FABMS and to Prof P Pachaly (Pharmazeutisches Institut der Universitat, Bonn, F R G) for HRMS

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