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GLYCORIC ACID POSSESSING A NEW 10-NORMEGASTIGMANE SKELETON FROM *GLYCOSMIS ARBOREA*

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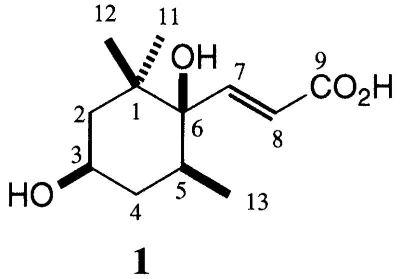
Glycoric acid (**1**) belonging to a new 10-normegastigmane skeleton was isolated from the hepatoprotective *n*-butanol soluble fraction of the methanol extract of *Glycosmis arborea* and characterized as (7*E*,3*R**,5*S**,6*R**)-3,6-dihydroxy-10-normegastigm-7-en-9-oic acid on the basis of 2D NMR and other spectral analyses.

KEY WORDS glycoric acid; 10-normegastigmane; *Glycosmis arborea*; Rutaceae

Glycosmis arborea (Roxb.) DC. (Rutaceae), an indigenous plant in India, is locally used against fever, liver complaints and certain other diseases.[1)] Preliminary pharmacological investigation on the *n*-butanol soluble fraction of the methanol extract of the overground part of the plant showed[2)] significant hepatoprotective activity against CCl_4-induced liver toxicity in experimental animals. With a view to searching for hepatoprotective principle(s), we undertook chemical investigation of this fraction and we isolated a very polar compound, designated as glycoric acid (0.0003%) and characterized it as (7*E*,3*R**,5*S**,6*R**)-3,6-dihydroxy-10-normegastigm-7-en-9-oic acid (**1**) on the basis of detailed 2D NMR and other spectral analyses. Although a number of natural products belonging to the megastigmane skeleton have been reported[3)] from plant sources, **1** seems to be the first representative of the 10-normegastigmane skeleton isolated from a natural source. Herein we report the structure elucidation of **1**.

The high-resolution mass spectrum of **1**, mp 268–270°C, $[\alpha]_D^{23}$ −24.3°C (*c*=0.5, MeOH); ν_{max}^{KBr} cm^{-1}: 3426, 3360, 2674, 2592, 1697, 1654; *m/z* (rel. int.) 228 (M^+, 1), 210 (16), 192 (5), 172 (52), 154 (65), 142 (40), 128 (100), 110 (22), 100 (12), 82 (19), showed the molecular ion at *m/z* 228.1344 corresponding to the molecular formula $C_{12}H_{20}O_4$. Its 500 MHz ^1H NMR spectrum (Table 1) revealed the presence of a *trans* –CH=CH– grouping and a $–CH_2–CH(OH)–CH_2–$ moiety with the OH group equatorially oriented, in addition to one secondary and two tertiary methyl groups. The ^{13}C NMR spectrum (Table 1) of the compound showed, besides the signals for the above mentioned groups, a singlet at δ 170.26 ppm assignable to an α,β-unsaturated CO_2H carbon and a singlet at δ 78.90 ppm for



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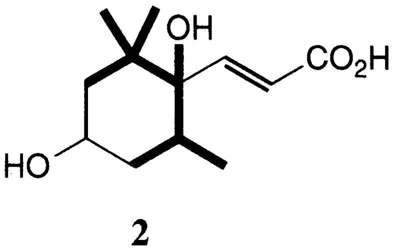

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Table 1. ^1H and ^{13}C Chemical Shifts (CD_3OD, TMS), and HMBC Data of **1***

δ_H ppm	δ_C ppm	Two- and three-bond ^1H-^{13}C correlation , δ_C ppm			
1.023 (H_3-11)	25.15q (C-11)	25.94q (C-12)	40.85s (C-1)	45.73t (C-2)	78.90s (C-6)
0.868 (H_3-12)	25.94q (C-12)	25.15q (C-11)	40.85s (C-1)	45.73t (C-2)	78.90s (C-6)
0.809d (H_3-13) (*J*=6.7 Hz)	16.44q (C-13)	35.27d (C-5)	39.64t (C-4)	78.90s (C-6)	
1.666dd (H_{ax}-2) (*J*=12.0, 11.6 Hz)	45.73t (C-2)	25.15q (C-11) 67.27d (C-3)	25.94q (C-12)	39.64t (C-4)	40.85s (C-1)
1.418dd (H_{eq}-2) (*J*=12.0, 6.5 Hz)	45.73t (C-2)	39.64t (C-4)	40.85s (C-1)	67.27d (C-3)	78.90s (C-6)
3.823dddd (H_{ax}-3) (*J*=11.6, 11.6, 6.5, 4.6 Hz)	67.27d (C-3)	45.73t (C-2)			
1.381ddd (H_{ax}-4) (*J*=13.1, 11.6, 11.6 Hz)	39.64t (C-4)	16.44q (C-13)	35.27d (C-5)	45.73t (C-2)	67.27d (C-3)
1.701m (H_{eq}-4)	39.64t (C-4)				
2.058qdd (H_{ax}-5) (*J*=13.1, 6.6, 3.8 Hz)	35.27d (C-5)	16.44q (C-13)	39.64t (C-4)	67.27d (C-3)	154.32d (C-7)
6.893d (H-7) (*J*=15.6 Hz)	154.32d (C-7)	35.27d (C-5)	78.90s (C-6)	123.31d (C-8)	170.26s (C-9)
6.056d (H-8) (*J*=15.6 Hz)	123.31d (C-8)	78.90s (C-6)	170.26s (C-9)		

*1D and 2D NMR spectra were recorded in a JEOL 500 MHz instrument. ^1H and ^{13}C signal assignments were done on the basis of ^1H–^1H COSY, ^1H–^{13}C COSY, HSQC and HMBC spectral analyses.

a quaternary carbon linked to a second OH group. The IR bands at 1697, 1654, 2592 and 2674 cm^{-1} and the UV maximum at 241.6 nm supported the presence of an α,β-unsaturated CO_2H group. The skeleton of the compound was determined by 2D NMR spectral analyses. Thus, from the two- and three-bond correlation of the methyl protons with other carbons (Table 1) obtained from the HMBC spectrum of **1**, the part structure shown by heavy lines in **2** could be easily determined. Further correlations of the protons attached to C-2 elaborated the part structure to the full ring system with the secondary OH group at C-3. Again, the correlations observed for the H-5, H-7 and H-8 proton signals clearly demonstrated that the –CH=CH–CO_2H moiety must be attached to C-6.

On the basis of the above observations, the (7*E*,3*R**,5*S**,6*R**)-3,6-dihydroxy-10-normegastigm-7-en-9-oic acid structure (**1**) was assigned for glycoric acid. The structure was fully supported by its mass spectral fragmentation pattern and all the fragment ions could be derived from the intermediate diradical (species *a*) obtained by rupture of the bond between C-1 and C-6 (Chart 1). Thus, while cleavage of the bond between C-4 and C-5 (path a) would result in the formation of ions at *m/z* 100 (species *b*) and *m/z* 128 (species *c*), cleavage through path b could lead to ion at *m/z* 142 (species *d*) and fragmentation through path c could form the ion peak at *m/z* 172 (species *e*). The other fragment ions at *m/z* 210, 192, 154 and 110 could be easily obtained by the elimination of a molecule of H_2O from their respective parent ions, *viz.* M^+, [M^+–H_2O], species *e* and species *c*.

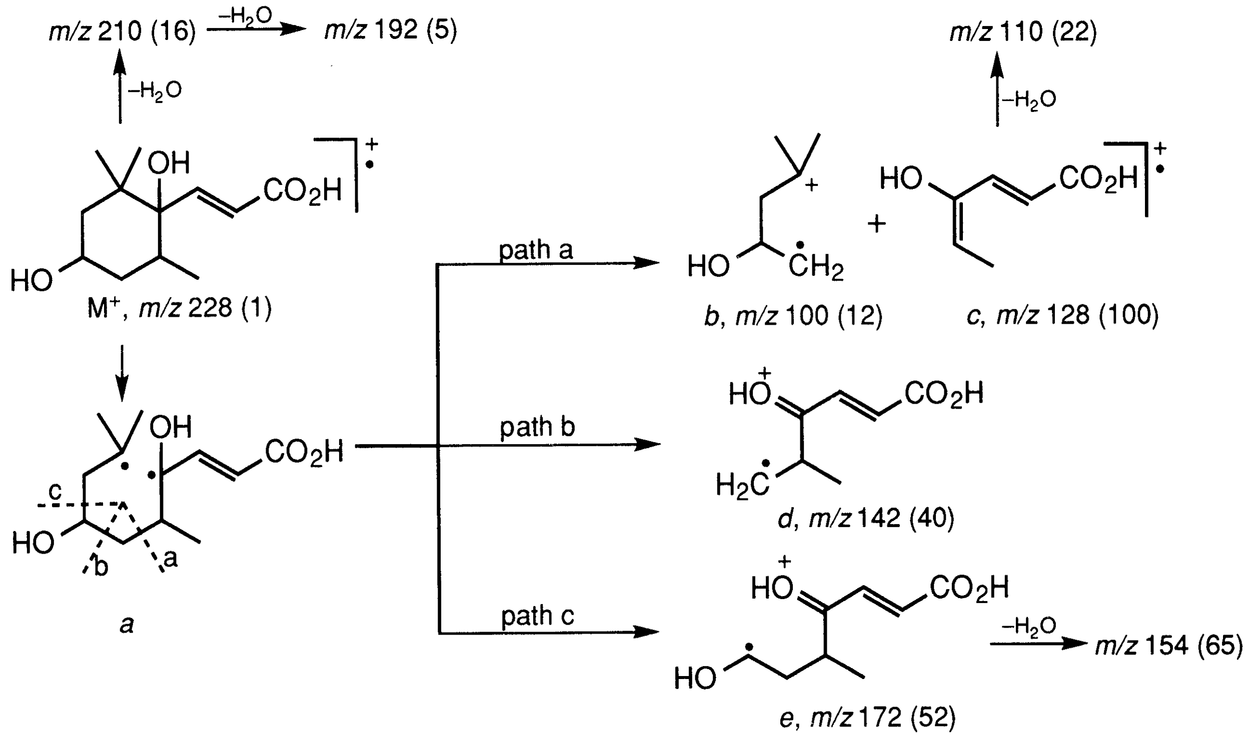

Chart 1

Finally, the relative stereochemistry of all the chiral centers of **1** were established from the NOE interactions observed in its NOESY spectrum, as depicted in Fig. 1. The absence of NOE interaction between H-7 and H_3-12 was fully substantiated by the long through-space distance (3.280 Å) between them as calculated using MM2 parameters.[4] The NOEs (Fig. 1) were observed only when the interproton distances ranged from 2.220 Å – 2.510 Å.

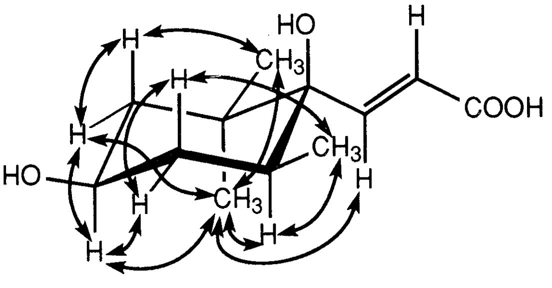

Fig. 1. NOE Interactions Observed in the NOESY Spectrum of **1**

Biogenetically, the 10-normegastigmane skeleton can be derived by the oxidative cleavage of the Δ^9 double bond of carotenoids.

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