

Note

Two new limonoids from the seed of
Microula sikkimensis H.

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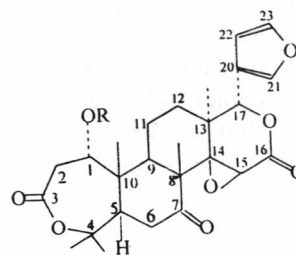
Received 6 September 1996; accepted 6 February 1997

Two new limonoids, named as microulin A **2** and microulin B **3**, have been isolated along with three known limonoids and their structures have been deduced on the basis of their spectral data. This is the first report on the isolation of limonoid from the seed of *Microula sikkimensis* H.

Microula sikkimensis H. contains seed oil up to 45%. It was reported that this oil has a strong effect on reducing triglyceride in serum¹, but very little is known about its chemical constituents except the analysis of the fatty acids and sterols^{2,3}. We have made a detailed investigation of the seed and isolated two new limonoids along with three known compounds.

The known compounds were identified by comparing their spectral data with those of authentic samples or with those reported in literature as: nomilin **1**⁴, rutaevin **4**⁵ and methyl deacetylnomilinate **5**⁶.

Microulin A **2** was obtained as white needles, m.p. 263-64°C (CHCl₃-CH₃COCH₃), analysed for C₃₃H₃₆O₁₀ by high resolution mass spectrometry. It gave the characteristic limonoid colour with Ehrlich reagent⁷. Its IR spectrum showed the absorptions of δ-lactone (1745 cm⁻¹), β-furan (1510, 875 cm⁻¹),



1 R=Ac

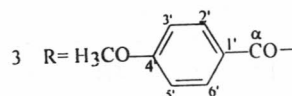
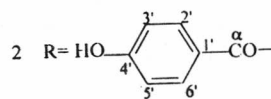


Fig. 1—The structures of compounds 1,2 and 3.

epoxide (1285 cm⁻¹), hydroxyl (3450 cm⁻¹) and aromatic ring (1610, 810, 770 cm⁻¹). The ¹H NMR spectrum of **2** was quite similar to that of nomilin **1** except in ring A (see Table I). All these evidence suggested that **2** has a similar skeleton to nomilin **1**. The difference of the ¹H NMR spectral data between **2** and **1** were that the former was lacking the signal of acetate methyl, but showed the signals at δ 9.70 (1H, s, -OH) and δ 6.93, 7.93 (4H, dd, J=8 Hz and 4 Hz, AA'BB' system), the chemical shifts and coupling constants of the latter two doublets indicated the presence of 1,4-disubstituted benzene ring. The mass spectrometry (FAB-MS) displayed a

Table I—¹H NMR chemical shifts of nomilin **1**, microulin A **2** and microulin B **3** in CDCl₃

Proton	1	2	3	Proton	1	2	3
H-1	4.98	5.36	5.30		1.45	1.46	1.45
H-2	2.15	2.20	2.20		1.32	1.30	1.31
H-6	2.03	2.00	2.00		1.08	1.02	1.02
H-15	3.78	3.82	3.80		1.18	1.10	1.10
H-17	5.42	5.40	5.40	-OCOCH ₃	1.98		
H-21	7.38	7.37	7.37	Ar-H		6.93	6.80
H-22	6.32	6.31	6.31			7.93	7.91
H-23	7.38	7.37	7.37	-OH		9.70	
-CH ₃	1.55	1.40	1.37	-OCH ₃			3.86

Table II— ^{13}C NMR chemical shifts of nomilin 1, microulin A 2 and microulin B 3 in CDCl_3

Carbon	1	2	3	Carbon	1	2	3
C-1	70.8	68.7	68.0	C-20	120.2	120.2	120.2
C-2	35.3	34.0	33.7	C-21	141.1	141.0	141.0
C-3	169.2	168.0	167.8	C-22	109.7	109.7	109.7
C-4	84.3	84.5	84.0	C-23	143.3	143.0	143.1
C-5	51.5	50.0	50.0	C-Methyls	33.5	33.4	33.2
C-6	38.9	38.9	38.7		23.4	22.9	22.9
C-7	206.9	206.0	205.7		20.8	20.1	20.0
C-8	52.9	52.8	52.8		17.2	17.0	16.8
C-9	44.3	44.0	44.0		17.2	16.7	16.7
C-10	44.3	44.8	44.8	Acetate-			
C-11	16.5	16.4	16.5	-Methyl	20.8		
C-12	32.2	32.1	32.1	C- α	169.2	164.6	164.2
C-13	37.6	37.3	37.3	C-1'		127.5	126.3
C-14	65.6	65.0	65.0	C-2',6'		131.1	130.0
C-15	53.5	53.5	53.5	C-3',5'		121.7	118.9
C-16	166.9	166.7	166.9	C-4'		154.6	160.0
C-17	78.1	77.9	77.9	-OCH ₃			55.7

molecular ion peak at m/z 593 ($M^+ + 1$) and significant fragment at m/z 138 ($\text{C}_7\text{H}_6\text{O}_3$), the latter fragment showed the presence of hydroxybenzoic acid. These were supported by ^1H - ^1H homonuclear decoupling experiments, in which irradiation at δ 6.93 collapsed the doublet at δ 7.93 to a singlet and vice versa. In the light of these observations, the structure 2 was assigned to this compound, which was substantiated by its ^{13}C NMR spectral data (see Table II).

Microulin B 3, $\text{C}_{34}\text{H}_{38}\text{O}_{10}$, m.p. 260-62°C, was obtained as white crystals (CHCl_3 - CH_3COCH_3). It gave the usual limonoid furan and H-17 signals⁸. The spectral data of 3 indicated that it has the same skeleton as that of 2 (see Table II). The ^1H NMR spectrum of 3 showed two doublets at δ 6.71 and 7.90 (4H, dd, $J=8$ Hz and 4 Hz, AA'BB' system) and three-protons singlet at δ 3.80 (OCH₃). This observation along with the fragment at m/z 152 ($\text{C}_8\text{H}_8\text{O}_3$) in the mass spectrum showed that 3 has an ester side chain with a composition $\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{CO}-\text{O}$ -. The structure of 3 was also confirmed by its ^{13}C NMR spectral data (see Table II).

Experimental Section

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer, mass spectra on a MAT-112 mass spectrometer and IR and UV spectra on Perkin-Elmer-599B and Shimadzu UV-250 spectrometers, Melting points are uncorrected.

Collection of the plant seed. The seed of *Microula sikkimensis* H. were collected from Gansu Province of China, in August 1991; a voucher

specimen identified by Dr Lian Y S is preserved in the Herbarium of the Botany Department, Northwest Normal University, Lanzhou-730070, People's Republic of China.

Extraction and isolation. Crushed *Microula Sikkimensis* H. seed (1 kg, 30-40 mesh) were extracted three times with petrol (30-60°C) at reflux temperature, then the residue was extracted with EtOAc (10 L) at room temperature with occasional stirring for 2-3 weeks. The EtOAc extract was concentrated to a syrup (12.5 g) and then subjected to chromatographic separation on silica gel (250-300 mesh) column. The components of the mixture were eluted with EtOAc and with EtOAc:EtOH (20:1, 10:1, 5:1, 2:1), respectively. Rechromatography of selected fractions and purification using preparative TLC yielded five compounds: 1 (50 mg), 2 (38 mg), 3 (35 mg), 4 (72 mg) and 5 (45 mg).

Microulin A 2 ($\text{C}_{33}\text{H}_{36}\text{O}_{10}$), m.p. 263-64°C; IR (KBr): 3450, 1745, 1725, 1668, 1610, 1510, 1285, 875, 810, 770 cm^{-1} ; FAB-MS: m/z 593 ($M^+ + 1$), 454, 138, 121; ^1H NMR and ^{13}C NMR are listed in Table I and II.

Microulin B 3 ($\text{C}_{34}\text{H}_{38}\text{O}_{10}$), m.p. 260-62 °C; IR (KBr): 2980, 1750, 1709, 1660, 1603, 1500, 1225, 822, 780 cm^{-1} ; FAB-MS: m/z 607 ($M^+ + 1$), 454, 152, 135; ^1H NMR and ^{13}C NMR are listed in Table I and II.

Acknowledgement

This work was supported by the Natural Foundation of Gansu Province of China.

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