

CHEMICAL AND PHARMACEUTICAL STUDIES ON MEDICINAL
PLANTS IN PARAGUAY: STUDIES ON "ROMERO," PART 2MUNEHISA ARISAWA,* TOSHIMITSU HAYASHI, KAZUNOBU OHMURA, KATSUYOSHI NAGAYAMA,
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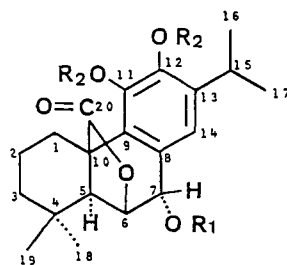
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"Romero" (*Rosmarinus officinalis* L., Labiatae) is a folk medicine in Paraguay (1). Earlier investigation of the constituents of this plant resulted in the isolation of rosmarinic acid (2). Afterwards, this plant afforded alkaloids (3-5), flavonoids (6,7), terpenoids (8,9), diterpene quinones (10), terpenic acids (11), carnosol (12), carnosic acid (13), and phenolic acid (14). Recently, several antioxidative constituents were isolated from the same plant (15-21). Previously, we reported biological studies for this medicine, including inhibitory activities against urease and KB cells (22).

In a continuing study of "romero," a new compound, 7-methoxyrosmanol [**1**], was isolated together with two known compounds, betulin and cirsimaritin. We wish to report the structural elucidation of the new compound.

The CHCl_3 extract was subjected to chromatographic separation which resulted in the isolation of the three compounds. Compound **1** was a pale brown powder, $\text{C}_{21}\text{H}_{28}\text{O}_5$, whose ir spectrum was similar to those of rosmanol [**2**] and 7-ethoxyrosmanol [**3**]. The ^1H -nmr spectrum of **1** was also similar to that of **2**, except for the appearance of the signals of the methoxy protons at δ 3.66 (3H) ppm (Table 1). Acetylation of **1** gave a diacetate [**1a**] that had a molecular ion at m/z 444 in the ms spectrum. The ^{13}C -nmr spectrum of **1** showed 21 carbon signals including a carbonyl carbon, 6 aromatic carbons, and 3 carbons attached to oxygen. A carbon signal of C-7 was lower than that of **2** by 9.3 ppm



	R ₁	R ₂
1	Me	H
1a	Me	Ac
2	H	H
3	Et	H

and 2 carbon signals of C-6 and C-8 were higher than those of 4.1 and 2.0 ppm, respectively (Table 2). From these spectral data, the structure of **1** was determined to be 7-methoxyrosmanol [**1**]. The remaining two compounds were identified as betulin and cirsimaritin by comparison with authentic samples. This is the first time these compounds have been isolated from this plant.

EXPERIMENTAL

PLANT MATERIAL.—Aerial parts of *R. officinalis* were collected in Cacupe, the suburbs of Asuncion, Paraguay, in July 1985. The voucher specimens are on deposit both in the institute of Toyama Medical & Pharmaceutical University and at Asuncion University.

EXTRACTION AND SEPARATION.—The dried "romero" (1.9 kg) was extracted three times with hot 70% EtOH. The EtOH extract (130 g) was partitioned between *n*-hexane and H₂O to afford *n*-hexane layer, H₂O layer, and precipitate. The H₂O layer was extracted with CHCl_3 . The CHCl_3 extract (14 g) was chromatographed on a Si gel column with elution by CHCl_3 - Me_2CO (200:1), followed by a second Si gel column with

TABLE 1. ^1H -nmr Spectral Data of Rosmanols (δ ppm, J =Hz)

Proton No.	Compounds			
	1 ^a	1a ^a	2 ^{b,c}	3 ^b
19-H	0.93 (3H, s)	0.93 (3H, s)	0.90 (3H, s)	0.91 (3H, s)
18-H	1.01 (3H, s)	1.01 (3H, s)	1.02 (3H, s)	1.02 (3H, s)
16-H	1.22 (d) (J =6.8)	1.18 (3H, d) (J =6.8)	1.17 (3H, d)	1.17 (3H, d)
17-H	1.22 (d) (J =6.8)	1.20 (3H, d) (J =6.8)	1.18 (3H, d)	1.20 (3H, d)
1 α -H	1.98 (1H, ddd) (J =13.7, 13.7, 5.5)	1.78 (1H, m)	1.98 (1H, dddd)	1.96 (1H, m)
5-H	2.24 (1H, s)	2.23 (1H, s)	2.29 (1H, s)	2.22 (1H, s)
15-H	3.06 (1H, sept) (J =6.8)	2.89 (1H, sept) (J =6.8)	3.27 (1H, sept)	3.27 (1H, m)
1 β -H	3.16 (1H, m)	2.83 (1H, m)	3.29 (1H, m)	3.30 (1H, m)
6 α -H	4.24 (1H, d) (J =3.2)	4.32 (1H, d) (J =2.9)	4.52 (1H, d)	4.35 (1H, d)
7-H	4.71 (1H, d) (J =3.2)	4.70 (1H, d) (J =2.9)	4.64 (1H, d)	4.75 (1H, d)
14-H	6.79 (1H, s)	7.23 (1H, s)	6.89 (1H, s)	6.84 (1H, s)
OAc		2.28 (3H, s) 2.30 (3H, s)		

^aIn CDCl_3 .^bIn acetone- d_6 .^cSee Inatani *et al.* (16).TABLE 2. ^{13}C -nmr Spectral Data of Rosmanols (δ ppm)

Carbon No.	1		2 ^a	3
	in CDCl_3	in acetone- d_6	in acetone- d_6	in acetone- d_6
1	27.2, t	28.1, t	28.4, t	28.3, t
2	19.0, t	19.8, t	19.9, t	19.8, t
3	38.0, t	38.9, t	39.1, t	38.9, t
4	31.5, s	31.9, s	32.0, s	32.0, s
5	50.8, d	51.3, d	51.0, d	51.5, d
6	74.6, d	74.7, d	78.8, d	75.4, d
7	77.5, d	78.4, d	69.1, d	76.8, d
8	126.4, s	128.0, s	130.4, s	128.4, s
9	124.2, s	124.8, s	124.9, s	124.1, s
10	47.0, s	47.5, s	47.7, s	47.6, s
11	142.4, s	144.5, s	144.7, s	144.6, s
12	142.1, s	142.6, s	142.5, s	142.6, s
13	135.1, s	136.2, s	136.5, s	136.3, s
14	120.5, d	120.6, d	120.2, d	120.6, d
15	27.2, d	27.3, d	27.5, d	27.4, d
16	22.3, q	22.8, q	22.9, q	22.9, q
17	22.5, q	23.1, q	23.1, q	23.1, q
18	31.4, q	31.9, q	31.8, q	31.8, q
19	22.1, q	22.3, q	22.4, q	22.3, q
20	179.0, s	178.5, s	178.3, s	178.4, s
OCH ₃	58.2, q	58.3, q		
OCH ₂ -				66.4, t
OCH ₂ CH ₃				16.2, q

^aSee Inatani *et al.* (16).

elution by C_6H_6 - Me_2CO (9:1) to afford crude **1** (34 mg), betulin (23 mg), and cirsimaritin (30 mg). The crude compounds were purified with plc.

CHARACTERIZATION OF 7-METHOXYXYROMANOL [1].—Pale brown powder; $[\alpha]^{25}_D -99.2^\circ$ ($c=0.5$, EtOH); positive to $FeCl_3$; uv λ max (EtOH) (log ϵ) 292 (3.24), 230 (4.00), 213 (4.27) nm; ir ν max ($CHCl_3$) 3500, 2975, 1740, 1440, 1360, 1215, 1090, 1040 cm^{-1} ; 1H nmr see Table 1; ^{13}C nmr see Table 2; ms m/z 360 (M^+), 316, 314, 284, 245, 215, 149; *Anal.* calcd for $C_{21}H_{28}O_5$: 360.1935. Found (ms): 360.1941.

ACETYLATION OF 1.—A mixture of **1** (5 mg), Ac_2O (0.1 ml), and pyridine (0.1 ml) was allowed to stand at room temperature overnight. The reaction mixture was worked up as usual to give a diacetate [**1a**] (4.5 mg). Negative to $FeCl_3$; 1H nmr see Table 1; ms m/z 444 (M^+), 402, 388, 361, 360, 346, 342, 316, 314, 258, 245, 215, 43; *Anal.* calcd for $C_{23}H_{32}O_7$: 444.2146. Found (ms): 444.2132.

IDENTIFICATION OF BETULIN.—Colorless needles, mp 248–250° (MeOH); $[\alpha]^{25}_D +11.63^\circ$ ($c=0.5$, $CHCl_3$). It was identified by comparison with published values (ir, 1H and ^{13}C nmr, ms, and $[\alpha]_D$) for betulin (23–26).

IDENTIFICATION OF CIRSIMARITIN.—Yellow needles, mp 264–268° (MeOH); uv λ max (MeOH) 331, 273 nm. It was identified by direct comparison with an authentic sample isolated from *Cirsium maritimum* Makino (Compositae) (27).

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