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THE STRUCTURES OF PULICARAL AND RELATED SESQUITERPENOIDS FROM *PULICARIA PALUDOSA*

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ABSTRACT.—The structures of pulicaral and other related [3.3.3] propellane sesquiterpenoids isolated from *Pulicaria paludosa* were determined by interpretation of their spectral data, mainly homonuclear and heteronuclear two-dimensional nmr correlations and nOe difference results.

Two-dimensional nmr spectroscopy has become one of the most useful techniques for the natural products chemist devoted to the structural determination of new metabolites. This is particularly true for substances containing rare or previously unknown skeletons (1,2), because in these cases little or no data for comparison are available. In the present paper we report how interpretation of some 1D- and 2D-nmr spectra, complemented with information obtained from other spectroscopic sources, permitted us to establish the constitution and stereochemistry of pulicaral and other related sesquiterpenoids isolated from *Pulicaria paludosa* Link (family Compositae; tribe Inuleae), a plant native to the western part of the Iberian peninsula. Previously, we reported the structural assignment of paludolon, another sesquiterpenoid with a novel skeleton, which was isolated from the same extract of this plant (3).

RESULTS AND DISCUSSION

Pulicaral is a crystalline compound, unstable in air and light, located in the medium-polarity fraction of the *n*-hexane extract of aerial parts of *P. paludosa*. Its mass spectrum showed a molecular peak $[M]^+$ at *m/z* 232.1431, in agreement with the molecular formula $C_{15}H_{20}O_2$ (calcd 232.1463). This was confirmed by its ^{13}C-nmr broad-band decoupled and DEPT spectra (Table 1, Figure 1), which revealed the existence in the molecule of three methyl groups (called **A**, **B**, and **D** for discussion in the text), four methylenes (**C**, **E**, **F**, and **G**), three methines (**H**, **M**, and **N**), and five nonprotonated carbon atoms (**I**, **J**, **K**, **L**, and **O**). The chemical shift of signals above 100 ppm revealed the presence of two carbonyl groups, one ketone (218.2 ppm) and one aldehyde (189.0 ppm, CH), as well as a trisubstituted double bond (143.8 ppm, =C<, and 159.5 ppm, =CH-) which must be conjugated with the aldehyde and must have substituents at both α and β positions from the aldehyde, because the protonated olefinic carbon was deshielded with respect to the nonprotonated one.

The above-mentioned four carbons support the whole functionality of the molecule, and because it was deduced from the molecular formula, pulicaral must have a three-ring skeleton. In addition, the existence of an α,β-unsaturated aldehyde in the molecule was confirmed by absorptions at 2720, 1690, and 1620 cm^{-1} in the ir spectrum (4) and by the absorption maximum at 241 nm ($\epsilon = 18900$) in the uv spectrum, which also agreed with the presence of a conjugated carbonyl (5). Furthermore, the ketone group absorbing at 1745 cm^{-1} in the ir spectrum must be located on a cyclopentane ring (6).

Apart from signals belonging to the aldehyde (9.73 ppm, 1H, s) and to the olefinic proton (6.43 ppm, 1H, s), the ^1H-nmr spectrum of pulicaral (Table 2, Figure 2) showed two methyl singlets at 1.16 and 1.06 ppm, one methyl doublet at 1.15 ppm, and a series of second-order overlapping multiplets between 2.5 and 1.3 ppm corresponding to nine protons. The interpretation of these latter multiplets was very difficult. Thus, to continue structural studies, some two-dimensional nmr experiments

TABLE 1. ^{13}C-nmr Data for Compounds **2–8**.[a]

Carbon	Compound						
	2	**3**	**4**	**5**	**6**	**7**	**8**
1	74.3	75.4	74.7	71.4	74.6	74.9	75.5
2	143.8	134.2	134.3	149.0	147.1	135.8	136.1
3	159.5	153.2	153.1	164.1	165.3	157.9	155.2
4	48.6	48.4	47.9	47.9	48.1	47.4	47.3
5	67.6	68.0	67.8	68.1	68.2	68.0	67.7
6	36.1	36.2	36.2	35.9[b]	35.3[b]	35.4	35.4
7	35.9	35.9	35.7	35.7[b]	34.7[b]	34.5	34.8
8	43.6	44.6	43.8	45.5	46.0	46.1	46.2
9	218.2	225.2	218.9	90.5	86.9	88.1	87.9
10	41.3	41.4	41.7	37.5	37.1	37.4	37.4
11	26.0	26.3	25.5	32.1	34.4	34.2	34.1
12	189.0	166.8	165.1	191.8	190.3	169.3	165.8
13	22.2	22.2	22.2	26.1[c]	24.5	24.7	24.7
14	26.5	27.1	26.9	26.2[c]	28.2	28.0	28.4
15	17.2	17.5	17.5	16.7	14.8	15.0	14.8
Other			51.4(OMe)				51.2(OMe)
				104.8	101.2	101.4	101.5
				76.6	73.4	73.5	73.6
				75.6	71.8	71.9	71.9
				74.2	71.6	71.7	71.7
				70.6	68.9	69.0	69.1
				62.6	62.2	62.3	62.3
					170.4	170.6	170.6
					170.1	170.3	170.3
					169.4(2)[d]	169.5(2)[d]	169.5(2)[d]
					20.8	20.8	20.9
					20.5(3)[d]	20.6(3)[d]	20.7(3)[d]

[a]$CDCl_3$; δ ppm from internal TMS.
[b,c]Assignments may be interchanged.
[d](2) = double intensity; (3) = triple intensity.

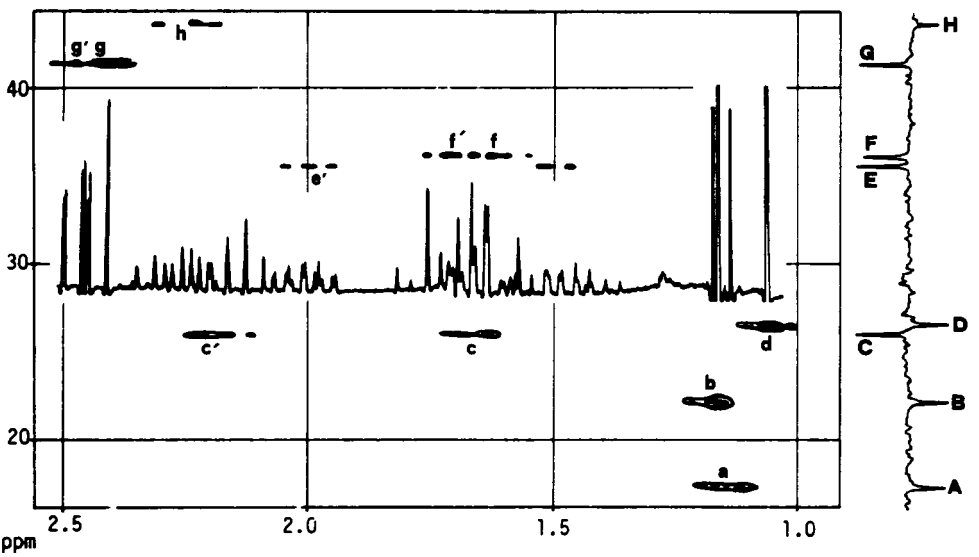


FIGURE 1. Up-field region of the one-bond ^{1}H-^{13}C correlation of pulicaral [**2**] with ^{1}H- and ^{13}C-DEPT spectra.

TABLE 2. ^{1}H-nmr Data for Compounds **2–8**.[a]

Proton	Compound				
	2	**3**	**5**	**6**	**7**
3	6.43 s	6.45 s	6.43 s	6.41 s	6.55 s
6	1.66 m	1.70 m			
7	2.00 m	2.07 m	1.3–2.0	1.3–2.0	1.3–2.0
	1.52 m	1.48 m			
8	2.28 dq	2.30 m			
9	—	—	3.83 bs	4.33 dd	4.41 bs
10	2.45 m	2.56 m			
11	1.60 m	1.60 m	1.3–2.0	1.3–2.0	1.3–2.0
	2.13 m	2.18 m			
12	9.73 s	8.70 bs	9.63 s	9.64 s	9.60 s
13	1.16 s	1.10 s	1.12 s	1.14[b] s	1.09[b] s
14	1.06 s	1.04 s	1.12 s	1.12[b] s	1.08[b] s
15	1.15 d	1.06 d	1.35 d	1.25 d	1.24 d
OMe		3.76 (**4**)			3.72 (**8**)
1′			4.25 d	4.69 d	4.67 d
2′					
3′			3.3–4.1	5.0–5.2	5.0–5.3
4′					
5′				3.68 ddd	3.69 ddd
6′			3.40 t	4.16 d AB	4.18 d AB
OAc				1.99	2.00
				2.02	2.02
				2.07(2)[c]	2.03
					2.07

[a]$CDCl_3$; δ ppm from internal TMS.
[b]Assignments may be interchanged.
[c](2) = Double intensity.

were conducted to establish connectivities of protons and carbons. Further, considering that ^{1}H homonuclear correlations were often unsuccessful for differentiating between geminal and vicinal couplings of second order 1D spectra, it was first necessary to obtain a one-bond ^{1}H-^{13}C heteronuclear correlation that would provide us with information about whether a certain proton was attached to a methine, a methylene, or a methyl carbon. Although the resolution in the H,C-COSY spectrum (7) (Figure 1) was not good, it was sufficient to locate intervals of absorptions for each proton and to relate these to their supporting carbons.

In this way analysis of the COSY spectrum (7) (Figure 2) was greatly simplified, and as shown in the figure the following correlations were detected: methyl doublet protons **a** connect with the methine proton **h**, which also correlates with methylene protons **e** and **e′** (the latter correlation was only observed in levels lower than those shown in Figure 2), and these two protons finally connect with **f** and **f′**. Furthermore, all possible connectivities between protons **c**,**c′** and **g**,**g′** are also observed. Eventually, due to the complexity of the absorption region of protons **f** and **f′** in the COSY spectrum, some correlation between **c** and **f** or **f′** or **e** protons could be suspected, although this is improbable since the **c′** proton shows no connectivity with **f**, **f′**, or **e′**, and the three couplings cannot be simultaneously zero due to bond angles.

Additional information from some long-range correlations observed in the COSY spectrum was obtained. Thus, apart from the expected **a**/**e**, **e′** long-range coupling, the correlations **d**/**c′**, **b**/**d**, and **b**/**c** (the latter detected only at a level lower than those

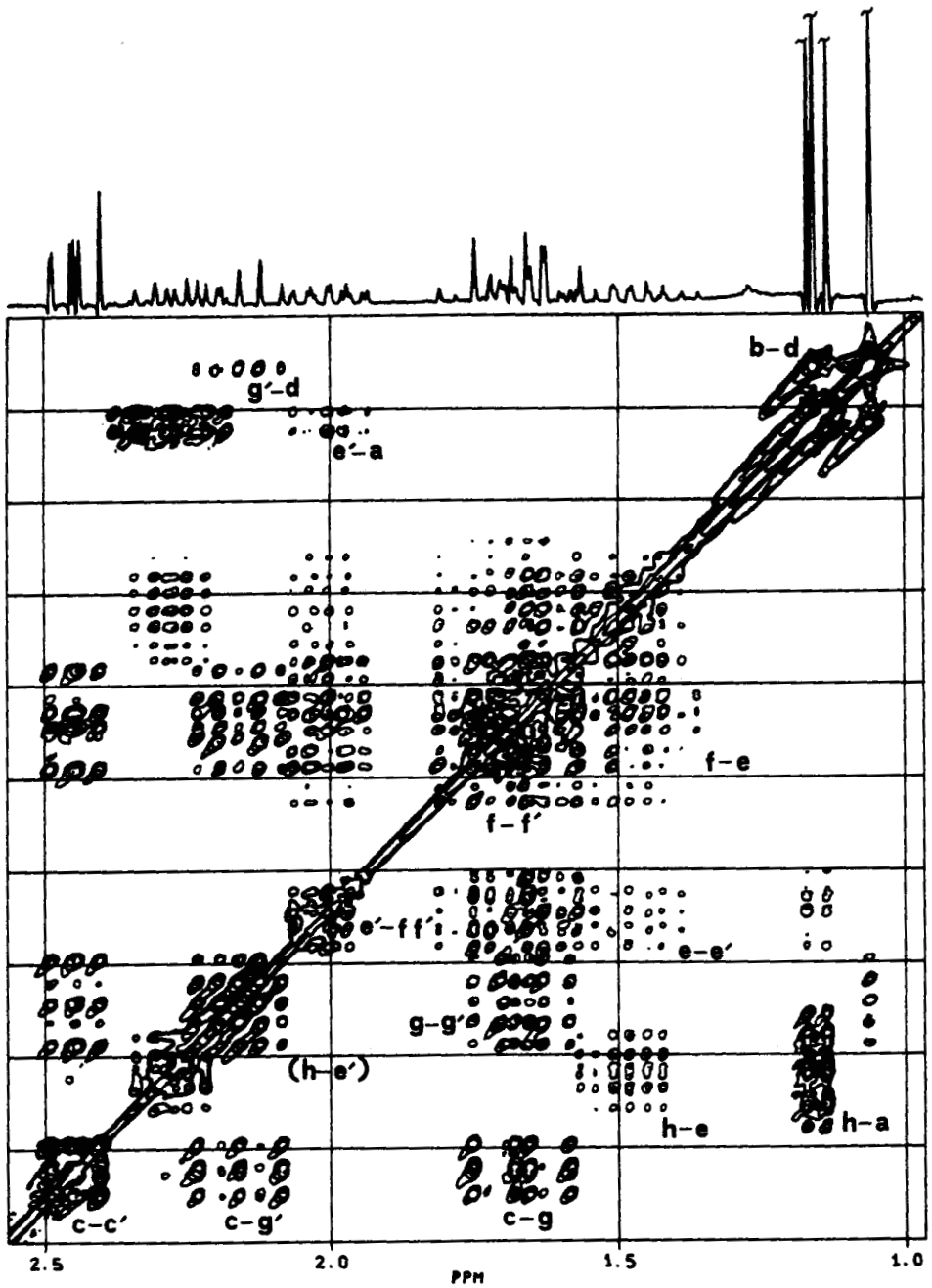


FIGURE 2. 200-MHz COSY-90 spectrum of pulicaral [**2**]. Bottom right: geminal and vicinal couplings; upper left: long-range connectivities.

represented in Figure 2) permitted us to deduce the proximity between methylene **c** and both methyl groups **b** and **d**. Furthermore, the **b** and **d** methyls must be geminal, as was confirmed by absorptions at 1365 and 1385 cm^{-1} in the ir spectrum (8).

Accordingly, the structure of pulicaral could be defined by a suitable arrangement on a tricyclic skeleton of the fragments depicted in Figure 3. Their actual combination was deduced from the analysis of a long-range H,C-COSY spectrum, optimized to search for $J_{C,H}$ couplings of about 7.8 Hz; the results are summarized in Table 3.

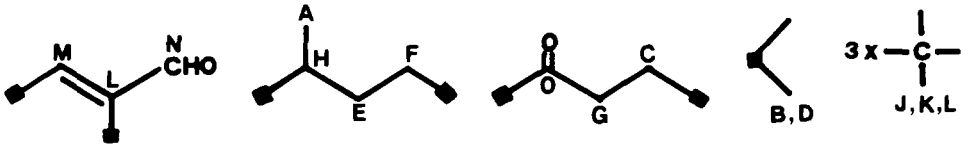


FIGURE 3. Structural fragments for pulicaral.

As can be seen, protons **b** and **d** of the *gem*-dimethyl group correlate with the olefinic carbon **M** and also with quaternary carbons **I** and **J**, whereas the remaining quaternary carbon **K** correlates with methyl protons **a** and with the olefinic proton **m**.

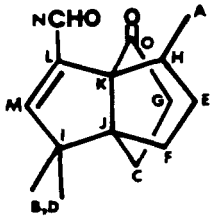
At this point, taking into consideration previous works on other species on the genus *Pulicaria* that have reported the occurrence of triquinane sesquiterpenoids in their extracts (9,10) and also bearing in mind that this kind of compound is often accompanied by caryophyllane derivatives, as in the case of *P. paludosa*, we propose structure **1** for pulicaral with a [3.3.3] propellane skeleton. The structure of modephene, the first natural sesquiterpene with such a skeleton, was established by Zalkow *et al.* (11) using X-ray diffraction methods, and apart from it, only one natural compound of this class has been described (12).

Our proposal was also supported by the analysis of relevant fragments in the mass spectrum of pulicaral, which were consistently interpreted as described in Scheme 1.

To confirm the constitution of pulicaral and to establish its relative stereochemistry, some nOe difference experiments were performed. Saturation of the resonance of

TABLE 3. Long-range H/C Connectivities for Pulicaral [2].

Carbon	Long-range connected protons
A	**e**
B	**d**
D	**b**
E	**a,f'**
G	**c'**
H	**a,f**
I	**b,d,c'**
J	**b,d**
K	**a,m**
L	**h,m,n**
M	**b,d**
N	**m**
O	**gg',c'**



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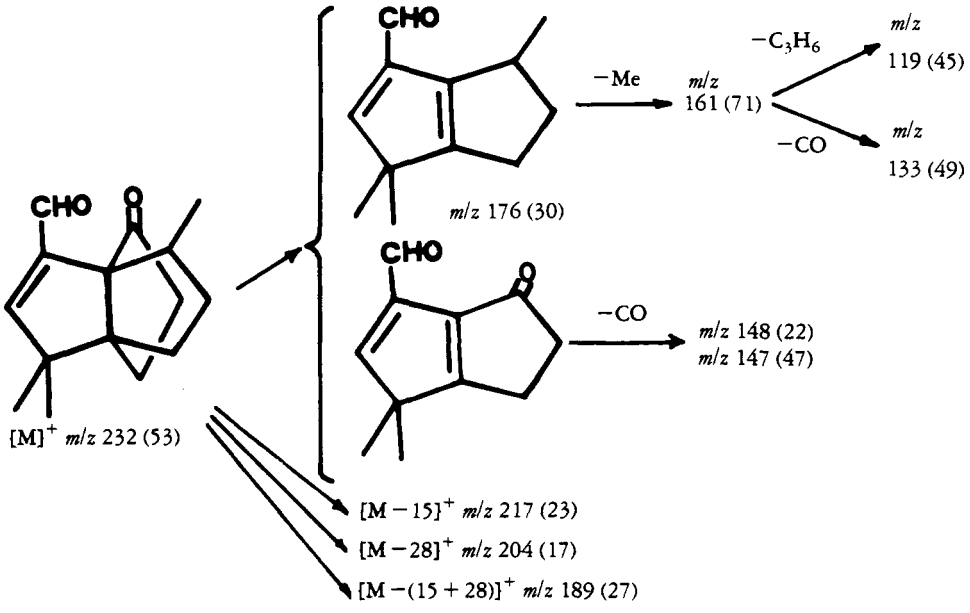
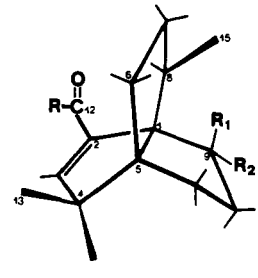


SCHEME 1

methyl protons **d** originated nOe over **g** (**g'**), **c'**, and **m**, whereas saturation of protons **b** produced nOe over **f** and **m**. This information proved that carbons **F** and **C** lie next to the *gem*-dimethyl group and are distant from the aldehyde; it furthermore served to assign unambiguously the signals belonging to both methyls.

The absolute stereochemistry of pulicaral has been assigned on the basis of cd studies. Because it was difficult to ensure a defined conformation for the α,β-unsaturated aldehyde because of free rotation about the **L–N** bond, the extended octant rule for β,γ-unsaturated ketones (13) was applied to assign the stereochemistry shown in **2**. The substance showed a negative Cotton effect at 294 nm, which defined a positive dihedral angle between the double bond and the ketone around the α–β bond (14,15). The configuration at carbon **H** was deduced from couplings of proton **h**, which determined the *pseudo*-equatorial conformation of methyl **A** and is in agreement with the probable biogenetic pathway of modephanes from (−)-β-caryophyllene (11,16), taking into account the absolute stereochemistry of caryophyllane derivatives isolated from the same extract (3) and from other species of the genus *Pulicaria*.



2 R=H; R_1,R_2=O
3 R=OH; R_1,R_2=O
4 R=OMe; R_1,R_2=O
5 R=R_1=H, R_2=β-D-glucoxyloxy
6 R=R_1=H, R_2=β-D-glucoxyloxy(2,3,4,6-tetraacetate)
7 R=OH, R_1=H, R_2=β-D-glucoxyloxy(2,3,4,6-tetraacetate)
8 R=OMe, R_1=H, R_2=β-D-glucoxyloxy(2,3,4,6-tetraacetate)

From the more polar fractions of the same extract, three additional substances structurally related to pulicaral were also isolated.

Substance **3** was an acid. Major differences from pulicaral in its ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) were consistent with the existence of a carboxyl group instead of the aldehyde in pulicaral. It is called pulicaric acid, and its methyl ester **4** has been prepared by methylation with ethereal CH_2N_2.

Compared with pulicaral, substance **5** showed in its ir spectrum intense bands of hydroxyl groups (3400, 1100–1050 cm^{-1}) and also the absence of the ketone band at 1745 cm^{-1}. The existence of complex absorptions in the 3.2–4.3 ppm region in its ^{1}H-nmr spectrum (Table 2), as well as six additional signals ranging from 62.6 to 104.8 ppm in its ^{13}C-nmr spectrum (Table 1), prompted us to assume the presence of a sugar which must be β-glucose as indicated by the absorption patterns shown in the nmr spectra of **5** and of its acetylated derivative **6**.

Substance **7** was isolated as its tetraacetate derivative. Comparison of its spectral data with those of **5** readily showed they differed by the existence of the carboxyl function in **7**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined in capillaries on a Buchi 510 instrument and are uncorrected. Optical rotations were measured in $CHCl_3$ on a Perkin-Elmer 241 digital polarimeter. Uv spectra were recorded in EtOH on a Hitachi 100-60 spectrometer. Ir spectra were obtained on a Beckman AccuLab VIII spectrophotometer in film or $CHCl_3$ solution. ^{1}H-nmr (200.13 MHz) and ^{13}C-nmr (50.3 MHz) spectra were recorded on a Bruker WP 200 SY spectrometer. Eims were obtained on a KRATOS MS-50 spectrometer at 70 eV. Cd was measured in EtOH on a Jobin-Yvon Dichrograph III. Flash chromatography was run on Si gel (Merck 9385) in a Eyela EF-10 chromatograph.

PLANT MATERIAL, EXTRACTION, AND ISOLATION.—The plant material was collected in July 1985, at Parada de Rubiales, Salamanca, Spain. Voucher specimens are deposited in the Botany Department (register number SALAF No. 13230). Air-dried material (7.2 kg) was extracted in a Soxhlet apparatus with *n*-hexane for 9 h to give, after cooling for 20 h at −20°, 266 g (3.7% of the dry wt of the plant) of soluble *n*-hexane extract. After successive separation of the MeOH-insoluble fraction and those components giving clathrates with urea-saturated MeOH, 6.3 g (2.4%) of the acid fraction was obtained by extraction with aqueous NaOH. The components were isolated by repeated cc and purified by cc and/or crystallization yielding *p*-methoxyacetophenone, anisic acid, veratric acid, three flavonoids, **3** (20 mg) and **5** (785 mg) eluted with $CHCl_3$-MeOH (9:1), and, from the more polar fraction after acetylation, **7** (15 mg).

From 12.5 g of the neutral fraction (3) 381 mg of pulicaral [**2**], eluted with $CHCl_3$-MeOH (99:1), was isolated.

Pulicaral [**2**].—Mp 70° (CH_2Cl_2); $[\alpha]^{24}$ (λ nm) −58.9° (589), −61.6° (578), −63.4° (546) (c = 0.3); uv λ max 241 nm (ε = 18900); ir ν max 2720, 1735, 1690, 1620, 1465, 1380, 1265, 1245, 1170, 1120, 1075, 950, 940 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* (%) 232 (53), 217 (23), 204 (17), 189 (27), 176 (30), 161 (71), 147 (48), 133 (44), 119 (45), 105 (70), 91 (79).

Pulicaric acid [**3**].—Oil; $[\alpha]^{24}$ (λ nm) −57.6° (589), −60.1° (578), −65.8° (546) (c = 0.7); uv λ max 238 nm (ε = 2600); ir ν max 3600–2200, 1740, 1690, 1610, 1455, 1380, 1270, 1170, 1110, 1035, 940, 910, 850 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1. Esterification of 19.2 mg of **3** with CH_2N_2 yielded 15 mg of methyl ester **4** as an oil: $[\alpha]^{24}$ (λ nm) −24.4° (589), −25.1° (578), −27.3° (546) (c = 0.5); uv λ max 238 nm (ε = 5200); ir ν max 1730, 1460, 1440, 1290, 1270, 1180, 1110, 1010, 990, 940, 900 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* (%) 262 (55), 247 (6), 234 (40), 231 (30), 230 (100), 215 (16), 207 (36), 196 (25), 191 (24), 187 (21), 175 (22), 165 (22).

Glycoside **5**.—Oil; $[\alpha]^{24}$ (λ nm) −17.2° (589), −14.3° (578), −15.1° (546), −26.0° (436), −41.1° (365) (c = 0.3); uv λ max 243 nm (ε = 5900); ir ν max 3420, 2820, 2750, 1680, 1620, 1470, 1390, 1375, 1255, 1180, 1150, 1100, 1050, 960, 910, 880 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1. Acetylation of 764 mg of **5** with Ac_2O/pyridine yielded 806 mg of acetate **6** as an oil: $[\alpha]^{24}$ (λ nm) +8.2° (589), +8.2° (578), +8.3° (546) (c = 1.4); uv λ max 240 nm (ε = 4000); ir ν max 2820, 1750, 1680, 1620, 1460, 1365, 1310, 1240, 1170, 1130, 1035, 990, 940, 910 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* (%) 564 (1), 536 (1), 505 (3), 347 (2), 331 (97), 271 (15), 259 (5), 242 (6), 233 (32), 217 (63), 169 (100).

Glycoside **7**.—Oil; $[\alpha]^{24}$ (λ nm) +19.0° (589), +20.0° (578), +22.8° (546) (c = 0.4); uv λ max 226

nm ($\epsilon = 4900$); ir ν max 3500–2400, 2740, 1775, 1690, 1630, 1480, 1450, 1370, 1235, 1175, 1140, 1040, 1000, 950, 905, 770 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 1. Esterification of 20 mg of **7** with CH_2N_2 yielded 21 mg of methyl ester **8** as an oil; $[\alpha]^{24}$ (λ nm) +6.7° (589), +7.5° (578), +8.0° (546) ($c = 0.8$); uv λ max 230 nm ($\epsilon = 2700$); ir ν max 1765, 1715, 1640, 1470, 1445, 1375, 1260, 1180, 1115, 1040, 940, 920 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* (%) 594 (3), 563 (1), 470 (1), 411 (1), 347 (1), 331 (81), 271 (7), 263 (10), 247 (45), 231 (12), 215 (15), 169 (100).

2D-NMR EXPERIMENTS.—*Heteronuclear ^1H-^{13}C correlations (200/50.3 MHz).*—Pulse sequence XHDEPT.AU from the Bruker DISnmr 85 program library was used, and 128 FIDs of 144 scans, each with 1 sec recycle delay and incrementing t_1 from 3 μsec to 130 msec, were acquired on a 0.6 M solution of **2** at 32°. Spectral widths (SW_2 and SW_1) were selected covering the range from 15 to 47 ppm in F_2 and the range 0.3 to 2.8 ppm in F_1, respectively. Polarization transfer was tuned for a value of $^1J_{C,H} = 135$ Hz. After sine-bell filtration in both domains with one degree of zero-filling in F_1, a matrix of 256/1024 data points with digital resolution (DR) = 3.1 Hz/point in F_2 and 0.95 Hz/point in F_1 was obtained (Figure 1).

The long-range $^1H/^{13}C$ correlation was performed on the same sample. SW_2 covered the range from 5 to 225 ppm and SW_1 from 0.3 to 12.3 ppm with the F_1 carrier placed suitably to avoid folding of signals. Number of scans per FID was 240. Response was tuned for $^nJ_{C,H} = 7.8$ Hz. DR was 10.8 Hz/point in F_2 and 4.6 Hz/point in F_1 (Table 3).

Homonuclear ^1H-^1H correlation (200 MHz).—COSY.AU pulse sequence (DISnmr 85) was used, and 256 FIDs of 16 scans, each with 1 sec recycle delay and incrementing t_1 from 5 μsec to 525 msec (the delay between both 90° pulses) were acquired on a 50 mM solution of **2** at 24°. The spectral width was selected covering only upper field region (0.3 to 2.8 ppm). Fourier transform was performed after sine-bell filtration in both domains leading to a 256/1024 data point matrix with DR = 0.95 Hz/point, which was symmetrized (Figure 2).

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