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400 MHz Proton Nuclear Magnetic Resonance Study of Magydardienediol, a Diterpene from *Magydaris panacifolia*

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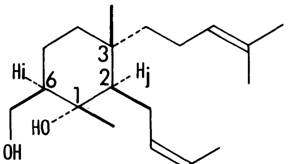
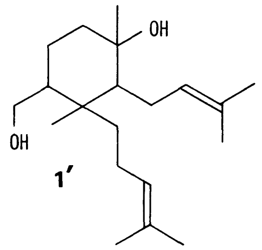
A detailed analysis of the 400 MHz ^{1}H-NMR spectrum of magydardienediol (**1**), a diterpene isolated from *Magydaris panacifolia* (Vahl) Lange, has been performed by use of a combination of proton homonuclear spin decoupling, COSY, NOE difference, and NOESY techniques. This ^{1}H-NMR spectral analysis, in conjunction with the mass spectral analysis of some derivatives of **1**, revealed that the structure of **1** should be revised to *t*-6-hydroxymethyl-1,*t*-3-dimethyl-*t*-2-(3-methyl-2-butenyl)-3-(4-methyl-3-pentenyl)-*r*-1-cyclohexanol.

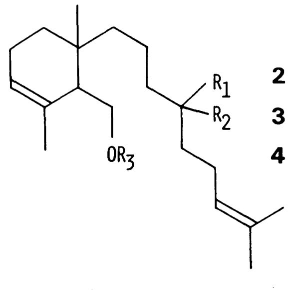
Magydardienediol, magydartrienol, and magydartrienyl acetate[1] are diterpenes isolated from *Magydaris panacifolia* (Vahl) Lange (Umbelliferae) and have been formulated as **2**, **3**, and **4**, respectively.[2] Recently, it was found that the ^{1}H-NMR spectral data of the diastereomers with structure **3** prepared from α-santonin were different from those reported for magydartrienol.[3] This finding prompted us to reinvestigate the structures of these natural diterpenes.[4]



1



1′



2 $R_1 = CH_3$ $R_2 = OH$ $R_3 = H$
3 R_1, $R_2 = CH_2$ $R_3 = H$
4 R_1, $R_2 = CH_2$ $R_3 = COCH_3$

We now wish to present a detailed analysis of the 400 MHz ^{1}H-NMR spectrum of magydardienediol (**1**) (Fig. 1) by use of a combination of ^{1}H-NMR spectroscopic techniques, *i.e.* proton homonuclear spin decoupling, two-dimensional proton-proton correlation (COSY)[5] (Fig. 3), NOE difference (Figs. 5 and 6), and two-dimensional NOE (NOESY)[6] (Fig. 7) spectroscopies, together with the mass spectral fragmentations of some derivatives of **1**. This spectral analysis showed unequivocally that the structure of magydardienediol should be revised to *t*-6-hydroxymethyl-1,*t*-3-dimethyl-*t*-2-(3-methyl-2-butenyl)-3-(4-methyl-3-pentenyl)-*r*-1-cyclohexanol (**1**),[7] and corroborated the structural revision of this diterpene preliminarily reported very recently by one of us and his associates.[8]

Magydardienediol (**1**), mp 55—56 °C (from hexane); $[\alpha]^{24}_{D}$ +24.0° (*c* 1.2, $CHCl_3$); EI-MS (*m/z* 308.2705. Calcd for $C_{20}H_{36}O_2$: M^+, *m/z* 308.2714); ^{13}C-NMR (see Experimental), is a monocyclic diterpene posessing two trisubstituted double bonds and two hydroxyl groups (one primary and one tertiary).[2] Its ^{1}H-NMR spectrum at 400 MHz (Fig. 1) showed distinctly four olefinic methyl peaks at δ 1.71, 1.70, 1.68, and 1.60; this indicated that the structure of **1** was quite different from that reported previously.[2]

It was found firstly that **1** had partial structures **A**, **B**, and **C**, along with two tertiary methyls (partial structures **D** and **E**), by the proton homonuclear spin decoupling experiments in conjunction with the COSY analysis (Fig. 2).

Partial structure **A**. The resonances at δ 2.30 (H_e) and δ 2.05 (H_f) were assigned to the two nonequivalent allylic methylene protons spin-coupled to the olefinic proton observed at δ 5.30 (H_a). Although the methine proton H_j signal at δ 1.59 (dd, J=8.5 and 3 Hz) was partially (one of the four-lines) overlapped by the olefinic methyl peak (δ 1.60), the cross peaks, H_e—H_j, in the COSY spectrum (Fig. 3) indicated unambiguously the vicinal spin couplings between these protons. The methine group (CH_j) had two quaternary carbon neighbors, because the irradiation at δ 1.59 (H_j) changed only the signals at δ 2.30 (H_e) and 2.05 (H_f).[9] Long-range spin couplings were observed between H_a and the olefinic methyls (δ 1.71 and 1.70) in both the decoupling and the COSY experiments. The broader peak at δ 1.70 was assigned to the olefinic methyl *cis* to H_a by analogy with the preceding examples[10] and also by the NOESY spectrum.

Partial structure **B**. Another spin system $(CH_3)_2C$=$CH_bCH_gH_h$ was identified by spin decoupling. The methylene protons (H_n and H_o) hidden among other multiplets were revealed by irradiation of the equivalent allylic methylene protons (H_g and H_h, δ 1.91) and analysis of the COSY spectrum (Fig. 3). The multiplicities of H_n (δ 1.32, d, J=15 Hz) and H_o (δ 1.21, d,J=15 Hz) in the spin decoupled spectrum showed that the methylene group (CH_nH_o) was connected to a quaternary carbon atom.

Partial structure **C**. The starting point in the analysis of this spin system was the two nonequivalent methylene protons, (H_c, δ 3.78) and H_d (δ 3.50), of the hydroxymethyl group. For this analysis the COSY spectrum (Fig. 3) was very efficient and the spin network $HOCH_2CHCH_2CH_2$ was deduced as the sole spin system consistent with the observation of the cross peaks, H_c-H_d, H_c-H_i, H_d-H_i, H_i-H_p, H_p-H_k, H_p-H_m, H_p-H_l, H_k-H_l, and H_k-H_m, Extensive decoupling

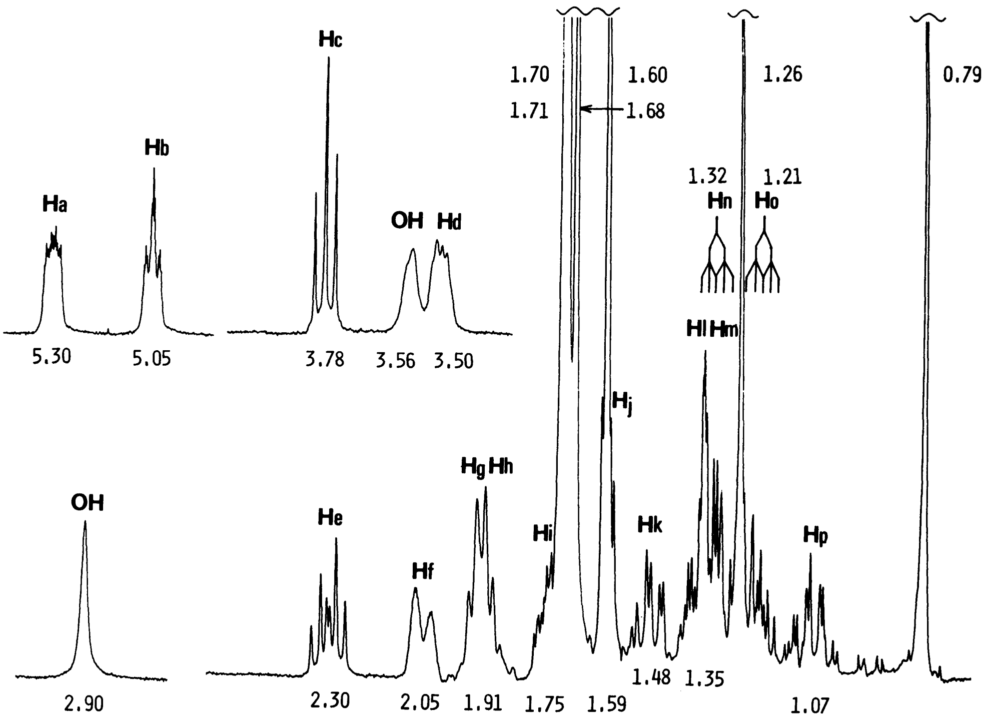


Fig. 1. 400 MHz ^{1}H-NMR Spectrum of magydardienediol (1) in $CDCl_3$.

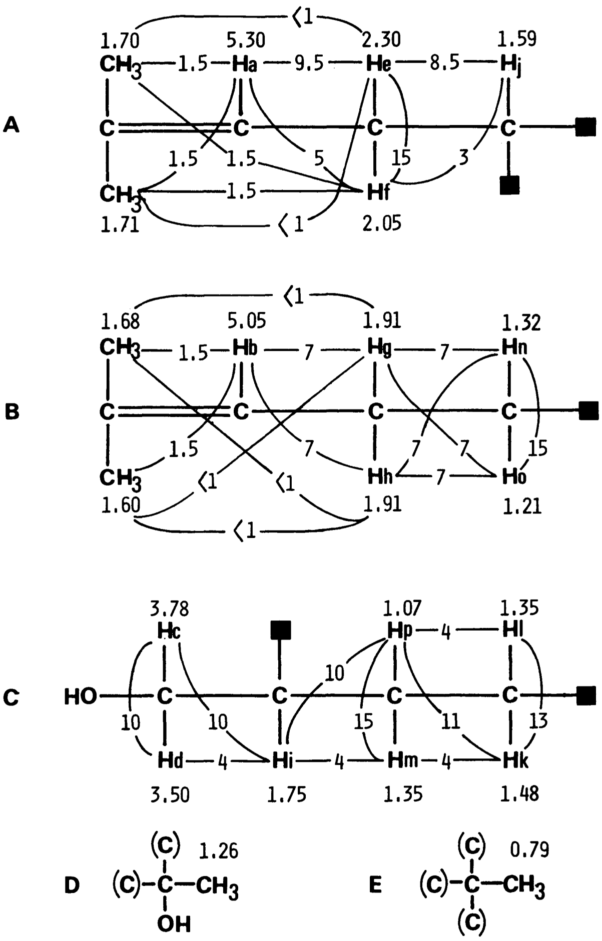


Fig. 2. Partial structures **A**—**E** and ^{1}H-NMR spectral data (chemical shifts (δ) and coupling constants (in Hz)).

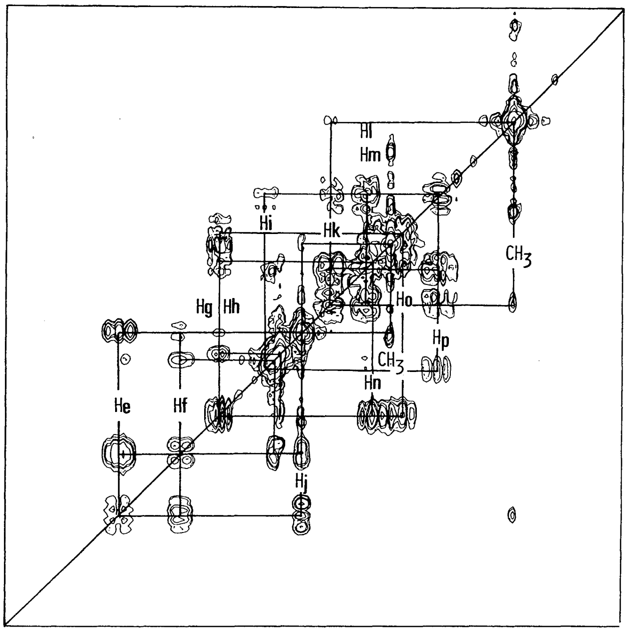
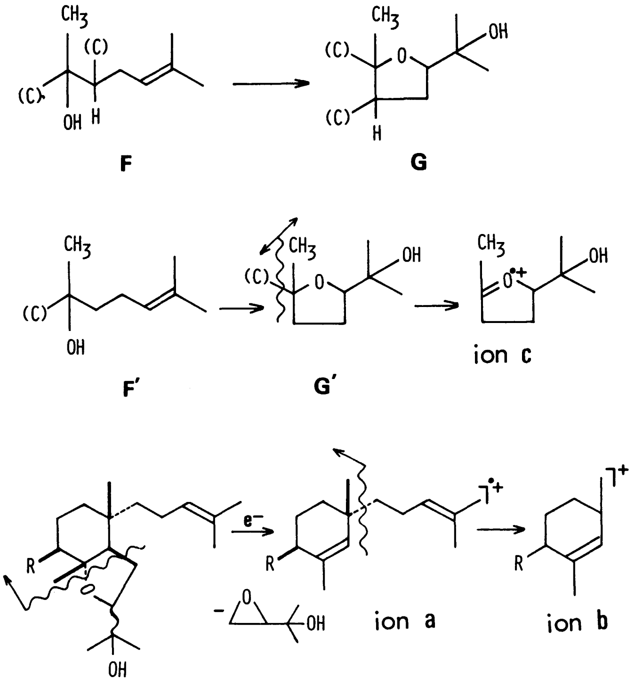


Fig. 3. Contour plot (high-field region) of the two-dimensional proton-proton correlation spectrum (COSY) of magydardienediol (1). The spectral width (total region) was 2000 Hz (1024×512 data points). Pulse delay time was 0.8 s and 16 times accumulation was used.

experiments revealed the multiplicities and the coupling constants of these protons. In particular, irradiation of H_l, (δ 1.35) and H_m (δ 1.35) observed in a highly congested region changed the signal patterns of H_p, (δ 1.07, dddd, J=15, 11, 10, and 4 Hz), H_k (δ 1.48, ddd, J=13, 11, and 4 Hz),[9] and H_i (δ 1.75, tt, J=10 and 4 Hz) into a double doublet (J=11 and 10 Hz), a doublet (J=11), and a triple doublet (J=10 and 4 Hz; two



5a **5b** R = CO_2CH_3

6a **6b** R = CH_2OH

Fig. 4. Mass spectral fragmentations of **5a**, **5b**, **6a**, and **6b**.

of the six-lines in higher field part were hidden among the olefinic methyls), respectively. The values of the geminal and vicinal coupling constants indicated that this grouping could be part of a five or six-membered ring system. The multiplicities of H_i and H_k suggested that both the methine (CH_i) and the methylene (CH_kH_l) groups had a quaternary carbon neighbor.

The following mass spectral analysis (Fig. 4) was very indicative of the connections among the spin systems (partial structures **A**—**E**) separated by the quaternary carbon atoms. Oxidation of magydardienediol (**1**) with Jones reagent, followed by methylation with diazomethane, gave a diastereomeric mixture (*ca.* 1 : 1) of tetrahydrofuran derivatives, **5a** and **5b**. The tetrahydrofuran ring moiety can be derived from either partial structure **F** (**A**+**D**) or **F′** (**B**+**D**).[2,11] Both the mass spectra of **5a** and **5b** exhibited two characteristic intense peaks at *m/z* 250 (ion **a** ($R=CO_2CH_3$)) and *m/z* 167 (ion **b** ($R=CO_2CH_3$)). The complete lack of the peaks at *m/z* 143 (ion **c**) and 209 ($[M-143]^+$) expected for partial structure **G′** disproved the presence of partial structure **F′** in **1**.[12] Ion **b** may be formed by cleavage of the tetrahydrofuran ring moiety followed by loss of the 4-methyl-3-pentenyl side chain.[13,14] Thus, it was shown that partial structures **F** and **B** were present in the diterpene (**1**).

From the foregoing evidence either **1** or **1′** could be deduced as the gross structure of magydardienediol. The diol (**1**) exhibited an IR absorption at $3520\,cm^{-1}$ ($5\times10^{-3}\,mol\,dm^{-3}$ in CCl_4) due to an intramolecular hydrogen bond.[15] As the hydroxymethyl group has already been shown to be equatorial (*i.e.* axial H_i), only **1** can take up a conformation in which the hydroxyl groups are sufficiently close in space for intramolecular hydrogen bonding. In further support of the structure, the proximity of the hydroxymethyl group to $C_{(1)}$-CH_3 was ascertained by the NOE experiments (Figs. 5(iii) and 7) and the long-range spin couplings between H_k and $C_{(3)}$-CH_3 and between H_j and $C_{(1)}$-CH_3 were shown in the COSY (Fig.3) and the proton-proton long-range COSY[16] spectra.

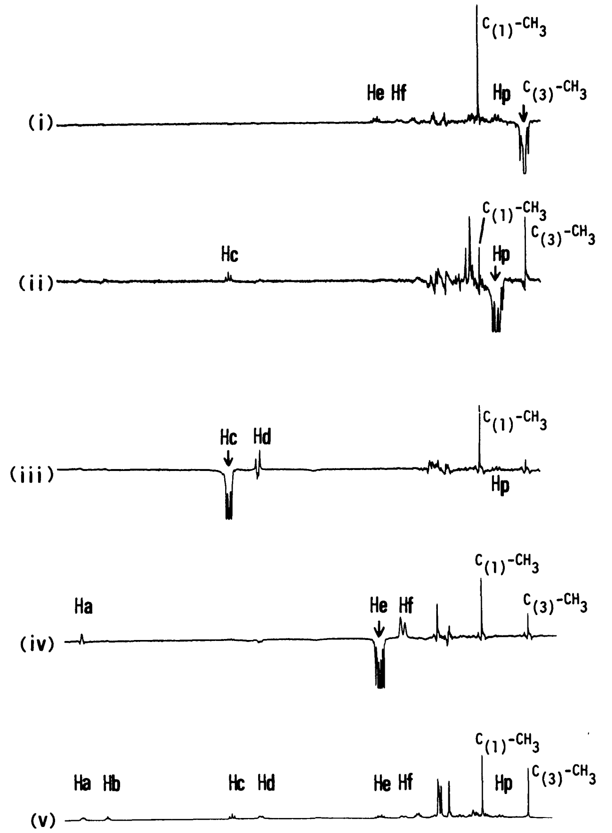


Fig. 5. Normal spectrum (v) and NOE difference spectra (i)—(iv) of magydardienediol (**1**). Irradiation protons are indicated by an arrow. The sum of the spectra obtained by irradiation of the downfield and the upfield components of H_e is shown in (iv).[17]

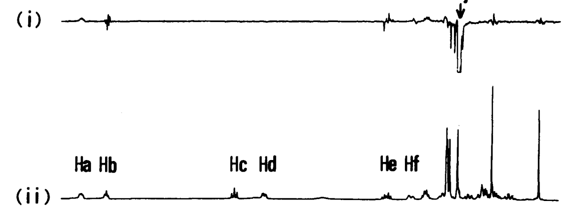


Fig. 6. Normal spectrum (ii) and NOE difference spectrum (i) of magydardienediol (**1**). Irradiation proton is indicated by an arrow.

The stereochemistry of magydardienediol (**1**) was established as that shown in Fig. 8 based on the NOE difference spectra and the NOESY spectral analysis (Fig. 7). When $C_{(3)}$-CH_3 was irradiated the $C_{(1)}$-CH_3 and the H_p signals were enhanced (Fig. 5(i)) and furthermore when H_p was irradiated both the peaks of the tertiary methyls were enhanced (Fig. 5(ii)); this indicated that the tertiary methyls and H_p were 1,3-diaxially oriented on a chair cyclohexane ring. The

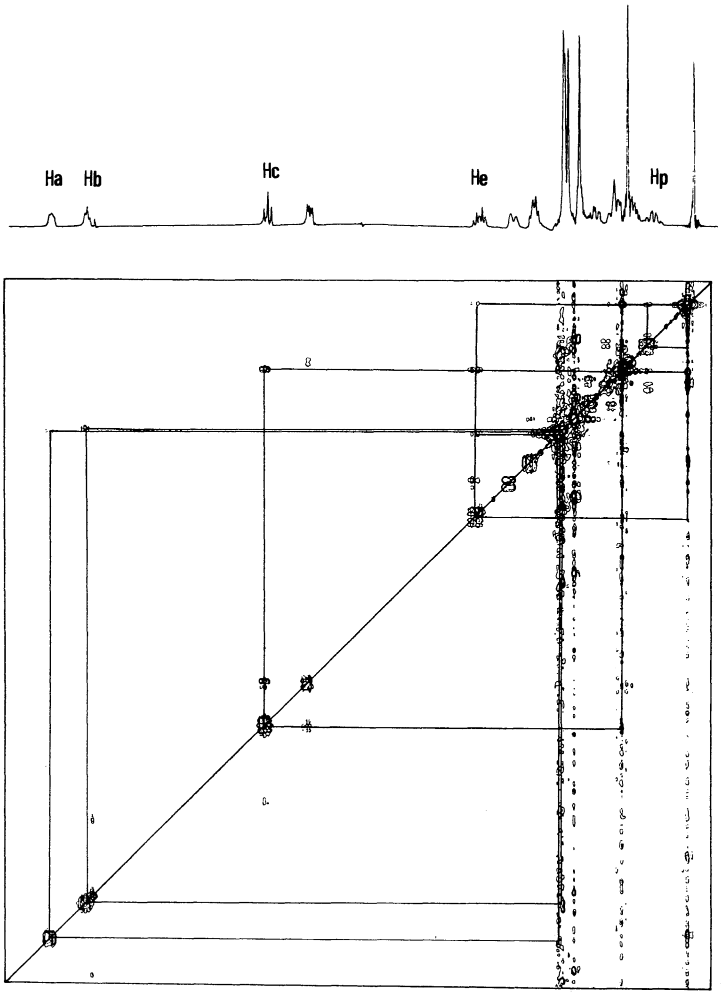


Fig. 7. Contour plot of the two-dimensional NOE spectrum (NOESY) of magydardienediol (**1**). The spectral width was 2000 Hz (1024×512 data points). Pulse delay and mixing times were 5 s and 1000 ms, respectively.

W-rule long-range spin coupling between $C_{(3)}$-CH_3 and the axial proton H_k (Fig. 3) gave additional evidence of the axial nature of the tertiary methyl.

As for the torsional isomerism about the single bond between the equatorial hydroxymethyl group and $C_{(6)}$, the intramolecular hydrogen bonding favored the conformation in which H_c and H_i were in antiperiplanar relation. This conformation was defined by the value of the vicinal coupling constant ($J=10$ Hz) observed between H_c and H_i and was well consistent with the NOE connectivities of H_c to $C_{(1)}$-CH_3 and H_p (Fig. 5(ii)(iii)).[15]

Finally, the *cis* relation of the 3-methyl-2-butenyl side chain to the tertiary methyls was revealed by the NOE experiments (Fig. 5(i) (iv)) which showed the proximity of H_e to the tertiary methyls. The axial nature of H_j was further shown by the W-rule long-range spin coupling between the axial $C_{(1)}$-CH_3 and H_j. The preferred conformation of the 3-methyl-2-butenyl side chain and the assignment of H_e and H_f were deduced as shown in Fig. 8 based on the NOE difference and NOESY experiments (Figs. 5(i) (iv), 6, and 7). The lower shift of the olefinic methyl resonance (δ 1.71) may be due to an anisotropy of the tertiary hydroxyl group in close proximity.[10]

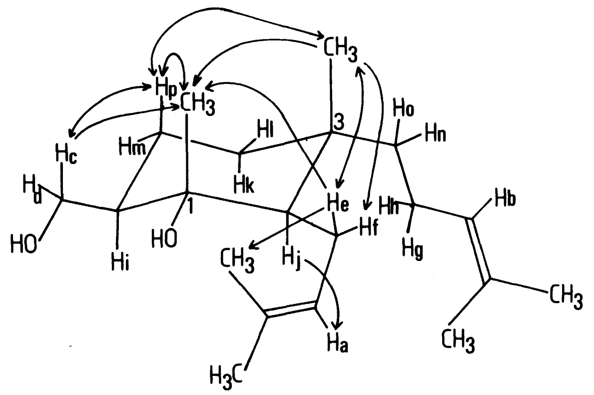


Fig. 8. Conformation and NOE connectivities of magydardienediol (**1**) (arrow heads show enhanced signals in the NOE difference spectra). The NOESY spectrum showed cross peaks corresponding almost to the above connectivities.

The structure of magydardienediol (**1**), a diterpene with a novel carbon skeleton, was thus established as *t*-6-hydroxymethyl-1,*t*-3-dimethyl-*t*-2-(3-methyl-2-butenyl)-3-(4-methyl-3-pentenyl)-*r*-1-cyclohexanol.

Experimental

Both ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL GX-400 spectrometer with tetramethylsilane as internal standard. Mass and IR spectra were obtained using JEOL DX-300 and JASCO A-3 spectrometers, respectively.

^{13}C-NMR Spectral Data of Magydardienediol (1) Derived from the Complete Decoupling and INEPT Spectroscopies (100 MHz, $CDCl_3$). δ 132.10 (C=), 131.30 (C=), 126.42 (CH=), 124.79 (CH=), 78.87 ($C_{(1)}$), 65.73 (CH_2OH), 54.81 (CH), 49.90 (CH), 43.81 (CH_2), 37.20 ($C_{(3)}$), 36.96 (CH_2), 25.91 (CH_3), 25.74 (CH_3), 23.92 (CH_2), 22.65 (CH_2), 22.00 (CH_2), 20.47 (CH_3), 19.11 (CH_3), 18.04 (CH_3), and 17.65 (CH_3).

*Preparation of **5a** and **5b**.* Magydardienediol (**1**) (15 mg) was treated with an excess of Jones reagent and the resulting carboxylic acids were methylated with diazomethane in ether. The crude product was chromatographed on silica gel (1 g) using hexane-ethyl acetate (3:1) as eluent to give a mixture of **5a** and **5b** (3 mg); one spot on TLC; IR spectrum of the mixture (neat): 3460 and 1730 cm^{-1}; GLC: retention times, 14.8 (**5a**) and 18.8 (**5b**) min (Carbowax 20M (20%, 2 m), 200 °C); GC-MS (Carbowax 20M (1.2 m), 70 eV) *m/z* (rel intensity) 352 (M^+; 0.7 (**5a**) and nil (**5b**)), 337 ($[M-CH_3]^+$; 1 (**5a**) and 1 (**5b**)), 334 ($[M-H_2O]^+$; 3.6 (**5a**) and 0.4 (**5b**)), 321 ($[M-OCH_3]^+$; 3 (**5a**) and 3 (**5b**)), 294 (12 (**5a**) and 15 (**5b**)), 250 (27 (**5a**) and 24 (**5b**)), 167 (41 (**5a**) and 30 (**5b**)), and 69 (100 (**5a**) and 100 (**5b**)); High-resolution mass spectrum of the mixture *m/z* 337.2369 (Calcd for $C_{20}H_{33}O_4$: *m/z* 337.2378), 250.1906 (Calcd for $C_{16}H_{26}O_2$: 250.1932), and 167.1069 (Calcd for $C_{10}H_{15}O_2$: 167.1071).

References

1) In the previous papers (Refs. 2 and 3) "magydar-2,13-diene-10,17-diol" and "magydar-2,10(20),13-trien-17-ol" were used for the names of magydardienediol and magydartrienol, respectively.

2) J. de Pascual Teresa, C. Grande, and M. Grande, *Tetrahedron Lett.*, **1978**, 4563.

3) H. Nagano, Y. Ishikawa, Y. Matsuo, and M. Shiota, *Chem. Lett.*, **1982**, 1947.

4) Our hope to establish the structures of these diterpenes by X-ray crystallography was frustrated by our inability to prepare suitable crystals of **1** or its *p*-bromobenzoate derivative (mp 69—71 °C, fine needles).

5) a) J. Jeener, *Ampère International Summer School*, Basko, Polje, Yugoslavia (1971); b) W. P. Aue, E. Bartholdi, R. R. Ernst, *J. Chem. Phys.*, **64**, 2229 (1976); c) A. Bax and R. Freeman, *J. Magn. Reson.*, **44**, 542 (1981).

6) S. Macura and R. R. Ernst, *Mol. Phys.*, **41**, 95 (1980).

7) Numbering of the cyclohexane ring reported in the previous paper (Ref. 8) was revised according to the IUPAC rules.

8) J. de Pascual Teresa, C. Grande, J. R. Moran, and M. Grande, *Chem. Lett.*, **1984**, 247.

9) Although the COSY (Fig. 3) and the proton-proton long-range COSY spectra showed furthermore the presence of long-range spin couplings between $C_{(1)}$-CH_3 and H_j and between $C_{(3)}$-CH_3 and H_k, their accurate coupling constants (<0.5 Hz) were not obtained.

10) R. B. Bates, R. H. Carnighan, R. O. Rakutis, and J. H. Schauble, *Chem. Ind. (London)*, **1962**, 1020; R. B. Bates and D. M. Gale, *J. Am. Chem. Soc.*, **82**, 5749 (1960).

11) E. W. Warnhoff and C. M. M. Halls, *Can. J. Chem.*, **43**, 3311 (1965).

12) C. R. Enzell, R. A. Appleton, and I. Wahlberg, "Biochemical Applications of Mass Spectrometry," ed by G. R. Waller, John Wiley, Chap. 13, p. 372.

13) Reduction of the mixture of **5a** and **5b** with lithium aluminium hydride gave a mixture of **6a** and **6b**, whose mass spectrum also exhibited intense peaks at *m/z* 222.1966 (52%, ion **a** ($R=CH_2OH$). Calcd for $C_{15}H_{26}O$: *m/z* 222.1983) and 139.1123 (40%, ion **b** ($R=CH_2OH$). Calcd for $C_9H_{15}O$: 139.1123). Catalytic hydrogenation (10% Pd-C, ethanol) of the mixture of **5a** and **5b** gave a mixture of dihydro derivatives and both the products exhibited a peak at *m/z* 167 (ion **b** ($R=CO_2CH_3$): 100%) in GC-MS analysis.

14) Catalytic hydrogenation (10% Pd-C, ethanol) of **1** gave a tetrahydro derivative, IR (neat) 3350 cm^{-1}; MS *m/z* 312.2929 (21%. Calcd for $C_{20}H_{40}O_2$: M^+, *m/z* 312.3027. Its mass spectrum (base peak, *m/z* 57) showed an intense peak (78%) at *m/z* 209.1859 (Calcd for $C_{14}H_{25}O$: *m/z* 209.1909) which must arise by dehydration of the molecular ion followed by loss of the 4-methylpentyl side chain.

15) A. Balsamo, P. Crotti, and F. Macchia, *J. Chem. Soc., Perkin Trans., 1*, **1982**, 3065.

16) Delay time (300 ms) was introduced at the beginning of the evolution and detection periods. Delay time between each pulse sequence was 0.8 s. (See Ref. 5c).

17) D. Neuhause, R. N. Sheppard, and I. R. C. Bick, *J. Am. Chem. Soc.*, **105**, 5996 (1983).