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2α,3β-DIHYDROXY-4(18)-*NEO*-CLERODEN-15-OIC ACID FROM *CISTUS POPULIFOLIUS*

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Key Word Index—*Cistus populifolius*; Cistaceae; diterpenes; *neo*-clerodanes.

Abstract—Besides the previously described diterpenes isolated from *C. populifolius*, one minor metabolite has been isolated and identified as methyl 2α,3β-dihydroxy-4(18)-*neo*-cleroden-15-oate. The structure of the new diterpene was confirmed by hemisynthesis.

INTRODUCTION

In previous papers on the chemical composition of *Cistus populifolius* L. a series of *neo*-clerodane diterpenoids was isolated and their structure established by means of spectroscopic techniques and thereafter confirmed by chemical transformations [1–4]. The requirement of the major components as homochiral† templates for the synthesis of possibly bioactive *neo*-clerodanes has prompted a new study of the acid fraction. We have now isolated a new *neo*-clerodane diol, **3**.

RESULTS AND DISCUSSION

In addition to the previously isolated major components **1** and **2**, from the more polar chromatographic fractions of the esterified Na_2CO_3 soluble part of the acid fraction obtained from a hexane extract of *Cistus populifolius*, a minor component **3** has been isolated, that showed in its IR spectrum strong absorption bands of hydroxyl groups (3400 cm^{-1}), a terminal double bond (3080, 1645 and 910 cm^{-1}) and an ester group (1735 cm^{-1}). The mass spectrum showed a parent molecular ion at *m/z* 352 corresponding to a molecular formula $C_{21}H_{36}O_4$. The data agree with the methyl ester of a bicyclic diterpene with a terminal double bond and two hydroxyl groups.

The ^{13}C NMR spectrum showed peaks corresponding to 21 carbon atoms. The protonated ones are sorted by DEPT subspectra as five methyls, seven methylenes (one of them sp^2 at δ101.7) and five methines. The four remaining ones are fully substituted, one being olefinic at δ158.0 and a carboxyl at δ173.6. The ^1H NMR spectrum showed as characteristic signals two singlets at δ4.97 and 4.76, respectively, assignable to the terminal double bond protons; the protons geminal to the hydroxyl groups appear at δ4.10 ppm (1H, *d*, *J* = 8.0 Hz) and δ3.23 (1H, *m*). Double irradiation experiments of the multiplet at δ3.23 cause the doublet at δ4.10 to collapse into a singlet, indicating that the two hydroxyl groups are located in contiguous positions. Moreover, the value of the coupling constant needs a great dihedral angle between the two hydrogens, suggesting that these have to be *trans* and equatorial. The methyl pattern: δ1.03 (3H, *s*), 0.90 (3H, *d*), 0.77 (3H, *d*) and 0.70 (3H, *s*) suggested a *neo*-clerodane skeleton. Because the ^{13}C NMR data indicated no substantial changes in ring B or in the side chain, the hydroxyl groups must be located at C-2 and C-3.

The final structure assignment and stereochemical determination of **3** was achieved by hemisynthesis (Scheme 1) from the major component **1**, whose structure was determined previously [1]. When **1** was treated with OsO_4 the diol **4** was obtained. Its acetylation and further elimination of the tertiary hydroxyl group led to the diacetyl derivative **6** that after hydrolysis and esterification gave the dihydroxy ester **3**.

EXPERIMENTAL

Spectral analysis. NMR: 200 MHz for ^1H and 50.3 MHz for ^{13}C. Chemical shifts are given in δ (ppm) and are referenced in $CDCl_3$ to the residual $CHCl_3$, 7.26 ppm for ^1H and 77.2 ppm for ^{13}C, respectively, unless otherwise stated. EIMS: VG TS 250 mass spectrometer, 70 eV. CC was carried out using silica gel Merck 60 (40–63 μm.).

Cistus populifolius L. was collected at Valle de las Batuecas (Salamanca, Spain). Air-dried material was extracted according to the description in ref. [1]. Beside the compounds previously isolated, the minor compound **3** was isolated from the more polar frs eluted with hexane–EtOAc (1:1).

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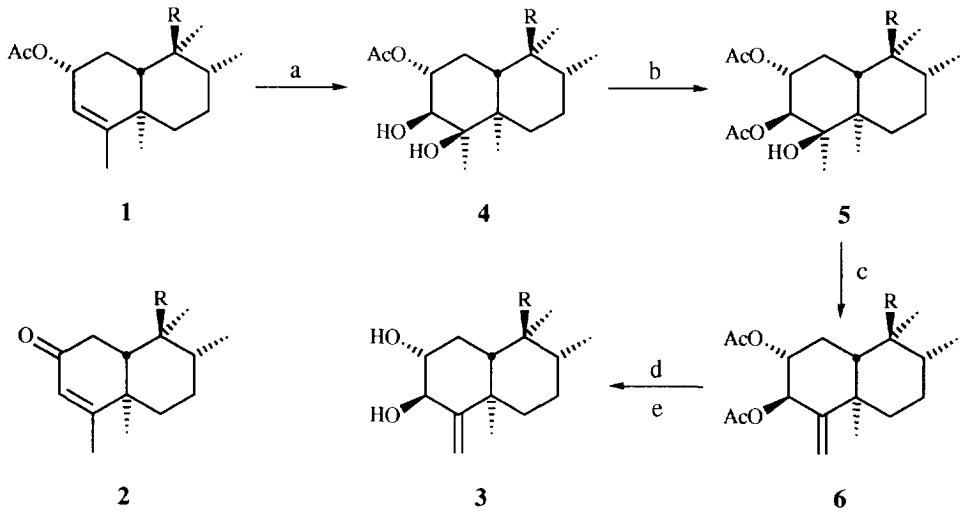
†The term homochiral has been defined by Davies, S. G. Brown, J. M., Pratt, A. J. and Fleet, G. (1989) in *Chemistry in Britain*, p. 260.



R = $-(CH_2)_2-CH(CH_3)-CH_2-CO_2Me$

Scheme 1. (a) OSO_4; (b)Ac_2O–pyridine; (c) $POCl_3$–pyridine; (d) KOH–MeOH; (e) CH_2N_2.

Methyl 2α,3β-*dihydroxy*-4(18)-neo-*cleroden*-15-*oate* (**3**). Oil. $[\alpha]_D$ − 7.3° ($CHCl_3$; *c* 1.0). EIMS, *m/z* (rel. int.): 352 $[M]^+$ (16.5), 337 (6), 257 (6), 206 (11), 175 (9), 137 (24), 123 (35), 109 (28), 99 (28.5), 83 (43.3), 69 (45), 40 (100). IR ν_{max}^{film} cm^{-1}: 3400, 3080, 2900, 1730, 1645, 1450, 1380, 910. ^1H NMR: δ4.97 (1H, *s*, H-18a), 4.76 (1H, *s*, H-18b). 4.10 (1H, *d*, *J* = 8.0 Hz, H-3), 3.66 (3H, *s*, COOMe), 3.23 (1H, *m*, H-2), 1.03 (3H, *s*, Me-19), 0.90 (3H, *d*, *J* = 6.0 Hz, Me-16), 0.77 (3H, *d*, *J* = 6.0 Hz, Me-17), 0.70 (3H, *s*, Me-20).

Treatment of **1** *with* OsO_4. *Methyl* 2α *acetoxy*-3β,4β *dihydroxy*-neo-*clerodan*-15-*oate* (**4**). To 87 mg (0.23 mmol) of **1** was added 31 mg (0.23 mmol) of N-methylmorfoline-N-oxide and 3.7 ml of *t*-BuOH–THF–H_2O (10:3:1). The mixt. was maintained under Ar and 0.05 ml of 2.5% OsO_4–*t*-BuOH was added. The reaction was left at room temp. for 53 hr and then it was chilled at 0° and 10 ml of satd soln of sodium sulphite added. After reaching room temp., stirring was maintained for 2 hr, the mixt. filtered over a celite pad, extracted with CH_2Cl_2, washed with H_2O, dried over Na_2SO_4, filtered and evapd to dryness affording 75 mg of crude product. This material was subjected to flash chromatography affording with hexane–EtOAc (1:1), **4** (55 mg, 58%). IR ν_{max}^{film} cm^{-1}: 3490, 2860, 1740, 1735, 1460, 1380, 1260, 1040, 1050, 960, 940. ^1H NMR: δ4.82 (1H, *m*, H-3), 3.55 (1H, *m*, H-2), 3.65 (3H, *s*, COOMe), 2.09 (3H, *s*, OCOMe), 1.17 (3H, *s*, Me-18), 0.95 (3H, *s*, Me-19), 0.91 (3H, *d*, *J* = 6.5 Hz, Me-16). 0.78 (3H, *d*, *J* = 6.5 Hz, Me-17), 0.69 (3H, *s*, Me-20). ^{13}C NMR: Table 1.

Acetylation of **4** *to yield methyl* 2α,3β-*diacetoxy*-4β-*hydroxy*-neo-*clerodan*-15-*oate* (**5**). Compound **4** (50 mg) was treated with Ac_2O in pyridine at room temp. to afford **5** (49 mg, 92.8%). ^1H NMR: δ5.15 (2H, *m*, H-2, H-3), 3.65 (3H, *s*, COOMe), 2.06 (3H, *s*, OCOMe), 2.00 (3H, *s*, OCOMe), 1.01 (3H, *s*, Me-18), 0.99 (3H, *s*, Me-19), 0.92 (3H, *d*, *J* = 6.5 Hz, Me-16), 0.75 (3H, *d*, *J* = 6.5 Hz, Me-17), 0.68 (3H, *s*, Me-20).

Table 1. ^{13}C NMR spectral data for **3**, **4** and **6** ([δ (ppm) in $CDCl_3$]

C	3	4	6
1	28.4	26.0	26.0
2	75.3	75.3	73.4
3	76.8	77.1	75.1
4	158.0	78.4	153.2
5	39.9	41.5	39.9
6	37.2	31.6	36.4
7	27.2	26.8	27.0
8	36.4	36.0	36.3
9	38.9	38.5	38.9
10	44.9	37.5	44.5
11	35.4	35.6	35.0
12	29.2	29.4	29.1
13	30.9	31.0	30.8
14	41.4	41.4	41.3
15	173.6	173.8	173.6
16	19.9	20.0	20.0
17	15.8	15.9	15.7
18	101.7	19.5	103.2
19	21.4	17.2	21.3
20	18.2	18.4	18.0
COOMe	51.4	51.4	50.4
OCOMe		172.1	170.1
OCOMe		21.4	21.4
OCOMe			170.1
OCOMe			21.2

Dehydration of **5** *to yield methyl* 2α, 3β-*diacetoxy*-4(18)-neo-*cleroden*-15-*oate* (**6**). To a soln of **5** (44 mg, 0.1 mmol) in pyridine (2 ml), cooled in an ice-bath was added

$POCl_3$ (0.3 ml). The mixt. was then heated at 100° for 10 hr. After cooling to room temp., ice was added and the mixt. extracted with Et_2O. After usual work-up, 31 mg of **6** (70%) was obtained, EIMS, *m/z* (rel. int.): 394 $[M]^+$ (2), 334 (100), 303 (6), 205 (27), 187 (27), 159 (6), 135 (10), 123 (16), 95 (16), 81 (8), 69 (17), 19 (17). IR ν_{max}^{film} cm^{-1}: 3080, 2900, 1730, 1645, 1450, 1380, 1250, 910. ^1H NMR: δ5.55 (1H, *d*, *J* = 8.0 Hz, H-3), 4.75 (2H, *m*, H-18), 4.67 (1H, *m*, H-2), 3.63 (3H, *s*, COOMe), 2.08 (3H, *s*, OCOMe), 2.01 (3H, *s*, OCOMe), 1.14 (3H, *s*, Me-19), 0.89 (3H, *d*, *J* = 6.0 Hz, Me-16), 0.77 (3H, *d*, *J* = 6.0 Hz, Me-17), 0.69 (3H, *s*, Me-20). ^{13}C NMR: Table 1.

Saponification and esterification of **6** *to yield methyl* 2α, 3β-*dihydroxy*-4(18)-neo-*cleroden*-15-*oate* (**3**). To 31 mg (0.07 mmol) of **6** was added a soln of 2 N KOH–MeOH (1 ml). The mixt. was left at room temp. during 3 hr with monitoring by TLC. After that time the solvent was removed *in vacuo*, H_2O was added and the soln acidified and then extracted with Et_2O. After usual work-up the crude product was esterified with CH_2N_2 affording 26 mg of diol **3**.

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