

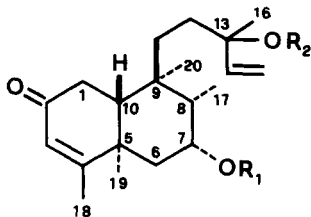
DITERPENES FROM *STACHYS RECTA*

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In connection with our interest in diterpene structure and synthesis (1), we have investigated the occurrence of a diterpene fraction in *Stachys recta* L. (Labiatae). By chromatography of the neutral fraction of the aerial parts of the plant, we have isolated three clerodane components, none of which had been found before in nature. They were identified as the 7,13-diacetate **1**, 7-acetate **2**, and 13-acetate **3** derivatives (2) of stachysolone **4**, a clerodane diterpene isolated (2, 3) from *Stachys annua*, whose configuration at C-8 and C-13 had not yet been assigned (4).

The structures of **1**, **2**, and **3** were mainly derived from spectral data (ms, pmr) and were confirmed by comparison of the physical constants (mp, rotation) with the values reported (2) for the acetate derivatives of **4**. Compounds **1**, **2**, and **3** were chemically correlated among themselves and to **4** by a simple series of hydrolysis and esterification reactions (see Experimental section for details).



	R ₁	R ₂
1	Ac	Ac
2	Ac	H
3	H	Ac
4	H	H

Configuration at C-8 was ascertained by performing nOe experiments with **1**. Irradiation of the 17-CH₃ doublet at δ 0.90 caused a significant enhancement of the methyl signal at δ 1.06, and vice-versa, thus showing close spatial prox-

imity between the two relevant methyl groups. Consequently, the methyl appearing at δ 1.06 is the 20-CH₃ and the 17-CH₃ is a α -oriented. (A *trans*-diaxial relationship would not have the observed nOes.) In addition, irradiation of the signal at δ 1.06 also caused the enhancement of the 1-CH₂ signals, confirming the assignment of the δ 1.06 signal to the 20-CH₃, and of the methyl signal at δ 1.29, which must be assigned to the 19-CH₃. Irradiation of the δ 1.29 methyl signal caused the expected enhancement of the δ 1.06 methyl singlet.

In view of the scarcity of ¹³C-data presently available for clerodane diterpenes (5), the ¹³C-spectra of compounds **1-4** have been recorded (see Table 1). The resonances have been assigned on the basis of the on- and off-resonance spectra and also by considering the changes in chemical shifts produced by acetylation (6) and the previous work (5) in this field, mainly that performed with 2-oxo-kolavenic acid (7). Only the assignments of the methylene lines deserve some comment. The C-1 signal was assigned by reference to 2-oxo-kolavenic acid and by considering that it should remain practically unchanged in all four compounds **1-4**. The same was assumed about C-11. The remaining two methylene carbon resonances (C-6 and C-12, both β -carbons to an hydroxyl or acetate grouping) fully agree with the values expected on the basis of acetylation effect.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.— Equipment used: mps: Kofler block; rotations, Perkin-Elmer model 141; ms, AEI model 902; nmr: Bruker, model WH270. The nuclear Overhauser effects were measured by difference FIDs with a microprogram virtually identical with the

TABLE 1. Cmr (67.88 MHz, CDCl₃) Chemical Shifts (δ)

Carbon Atoms	Compounds			
	1	2	3	4
C-1	34.73	34.61	34.76	34.69
C-2	199.59	199.88	200.27	199.87
C-3	125.22	125.32	125.09	125.03
C-4	171.82	171.89	172.78	172.76
C-5	39.07	39.04	39.33	39.35
C-6	38.60	38.62	41.38	41.43
C-7	74.57	74.60	73.22	73.30
C-8	37.74	37.69	38.68	38.77
C-9	38.13	38.01	37.95	37.89
C-10	45.46	45.49	45.69	45.84
C-11	31.74	32.18	31.83	32.35
C-12	33.27	35.06	33.17	35.09
C-13	82.76	72.99	83.30	73.08
C-14	141.71	144.81	141.68	144.87
C-15	113.66	112.66	113.46	112.17
C-16	23.65	27.82	23.43	27.70
C-17	11.89	11.90	12.26	12.32
C-18	18.95 ^a	18.97 ^b	19.03 ^c	19.02 ^d
C-19	19.35 ^a	19.33 ^b	19.70 ^c	19.67 ^d
C-20	20.02 ^a	19.94 ^b	20.24 ^c	20.18 ^d
CH ₃ COO-	21.42	21.41	22.22	
	22.22			
CH ₃ COO-	170.01	170.60	170.22	
	170.61			

a,b,c,d Interchangeable values.

one described in the Bruker Aspect 2000 NMR Software Manual.

ISOLATION OF 1, 2, AND 3 AND CHEMICAL CORRELATION.—Dried aerial parts (600 g) of *S. recta* (collected in the autumn in Toscana, Italy, and authenticated by the Botanical Garden of the University of Naples) were extracted with Me₂CO in a Soxhlet apparatus for 24 h. The evaporated extract (45 g) was dissolved in Et₂O and washed with 2 N NaOH and H₂O; the dried Et₂O solution was evaporated and the residue (8 g) chromatographed on a silica gel (320 g) column with C₆H₆ containing increasing amounts of Et₂O. Three main fraction groups were collected: *a* (630 mg, 9:1, C₆H₆-Et₂O); *b* (720 mg, 3:2, C₆H₆-Et₂O); and *c* (74 mg, 1:1, C₆H₆-Et₂O).

Chromatography of fraction *a* (silica gel column, C₆H₆-Et₂O) afforded a solid (360 mg). Crystallization from hexane yielded **1** (340 mg), mp 93–94°, [α]_D –68° (CHCl₃, *c*=1); ms *m/z* 404 (M⁺); pmr (270 MHz, CDCl₃) δ 0.90 (3H, d, *J*=7 Hz, 17-H₃), 1.06 (3H, s, 20-H₃), 1.29 (3H, s, 19-H₃), 1.51 (3H, s, 16-H₃), 1.56 (1H, m, 6-H_{ax}), 1.88 (3H, d, *J*=1 Hz, 18-H₃), 1.92 (1H, X of ABX, *J*_{AX}=13.5 Hz, *J*_{BX}=5.1 Hz, 10-H_{ax}), 2.03 (3H, s, OCOCH₃), 2.13 (3H, s, OCOCH₃), 2.21 (1H, dd, *J*_{α,β}=14.5 Hz,

*J*_{6α,7}=2.7 Hz, 6-H_{eq}), 2.36 (1H, B of ABX, *J*_{AB}=17.6 Hz, *J*_{BX}=5.1 Hz, 1-H_{eq}), 2.44 (1H, A of ABX, *J*_{AB}=17.6 Hz, *J*_{AX}=13.5 Hz, 1-H_{ax}), 5.12 (2H, AB of ABX, 15-H₂), 5.16 (1H, q, 7-H_{eq}), 5.70 (1H, bs, 3-H), 5.93 (1H, q, X of ABX, *J*_{AX}+*J*_{BX}=27.8 Hz, 14-H).

Plc (silica gel, 3:2, hexane-EtOAc, three runs) of fraction *b* gave pure **2** (85 mg), vitreous solid, [α]_D –73° (CHCl₃, *c*=0.7); ms *m/z* 362 (m⁺); pmr (270 MHz, CDCl₃) δ 0.91 (3H, d, *J*=7 Hz, 17-H₃), 1.05 (3H, s, 20-H₃), 1.27 (3H, s, 16-H₃), 1.29 (3H, s, 19-H₃), 1.50 (1H, dd, *J*_{6β,7}=3.7 Hz, *J*_{6α,β}=14.7 Hz, 6-H_{ax}), 1.88 (3H, d, *J*=1 Hz, 18-H₃), 1.93 (1H, X of ABX, *J*_{AX}+*J*_{BX}=17.6 Hz, 10-H_{ax}), 2.08 (3H, s, OCOCH₃), 2.21 (1H, dd, *J*_{6α,7}=3 Hz, *J*_{6α,β}=14.7 Hz, 6-H_{eq}), 2.40–2.45 (2H, AB of ABX, 1-H_{eq}, 1-H_{ax}), 5.14 (2H, AB of ABX, 15-H₂), 5.17 (1H, m, 7-H_{eq}), 5.70 (1H, bs, 3-H), 5.85 (1H, q, X of ABX, *J*_{AX}+*J*_{BX}=28.2 Hz, 14-H).

Fraction *c* was submitted to plc (silica gel, 3:2, hexane-EtOAc, three runs) yielding pure **3** (44 mg), mp 130–131° (from hexane), [α]_D –45.2° (CHCl₃, *c*=0.6); ms *m/z* 362 (M⁺); pmr (270 MHz, CDCl₃) δ 1.01 (3H, d, *J*=7 Hz, 17-H₃), 1.08 (3H, s, 19-H₃), 1.40 (3H, s, 20-H₃), 1.51 (3H, s, 16-H₃), 1.53 (1H, m, 6-H_{ax}), 1.88 (1H, m, 10-H_{ax}), 1.91 (3H, d, *J*=1 Hz, 18-H₃), 2.02

(3H, s, OCOCH₃), 2.19 (1H, dd, $J_{6\alpha,7}=2.6$ Hz, $J_{6\alpha,6\beta}=14$ Hz, 6-H_{eq}), 2.35 (1H, B of ABX, $J_{AB}=17.7$ Hz, $J_{BX}=4$ Hz, 1-H_{eq}), 2.45 (1H, A of ABX, $J_{AB}=17.7$ Hz, $J_{AX}=13.1$ Hz, 1-H_{ax}), 4.08 (1H, q, 7-H_{eq}), 5.12 (2H, AB of ABX, 15-H₂), 5.70 (1H, bs, 3-H), 5.94 (1H, q, X of ABX, $J_{AX}+J_{BX}=28.6$ Hz, 14-H).

Samples of **1** gave **2** by treatment with 5% methanolic KOH at room temperature for 30 min, and **4** by refluxing with 5% methanolic KOH for 2 h. A sample of **2** gave **4** by refluxing with 5% methanolic KOH for 2 h. A sample of **3** gave **4** in the same conditions. Samples of **4** gave **2** by acetylation with Ac₂O/Py (24 h, room temperature) and **1** by acetylation with AcCl/Py (24 h, room temperature). In all cases, the products were identified by tlc and pmr. Compound **4** obtained as above had mp 156-159° (from hexane-Et₂O) [lit. (8) 153-155°], [α]_D -29° (MeOH, c=0.8), [lit. (4) -25°]; ms *m/z* 320 (M⁺); pmr (270 MHz, CDCl₃) δ 1.02 (3H, d, $J=7$ Hz, 17-H₃), 1.08 (3H, s, 19-H₃), 1.28 (3H, s, 16-H₃), 1.40 (3H, s, 20-H₃), 1.54 (1H, m, 6-H_{ax}), 1.89 (1H, X of ABX, $J_{AX}=12.9$ Hz, $J_{BX}=4.4$ Hz, 10-H_{ax}), 1.91 (3H, d, $J=1$ Hz, 18-H₃), 2.20 (1H, dd, $J_{6\alpha,6\beta}=14$ Hz, $J_{6\alpha,7}=2.9$ Hz, 6-H_{eq}), 2.39 (1H, B of ABX, $J_{AB}=17.2$ Hz, $J_{BX}=4.4$

Hz, 1-H_{eq}), 2.45 (1H, A of ABX, $J_{AB}=17.2$ Hz, $J_{AX}=12.9$ Hz, 1-H_{ax}), 4.08 (1H, q, 7-H_{eq}), 5.13 (2H, AB of ABX, 15-H₂), 5.69 (1H, bs, 3-H), 5.82 (1H, q, X of ABX, $J_{AX}+J_{BX}=27.9$ Hz, 14-H).

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