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ISOTEUCRIN H_4, A 19-NOR-NEOCLERODANE DITERPENOID OF BIOGENETIC INTEREST FROM TEUCRIUM KOTSCHYANUM

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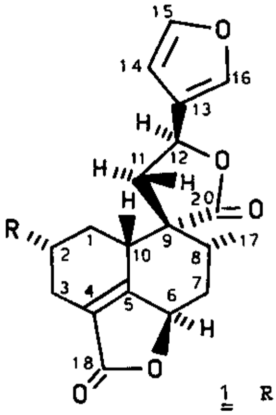
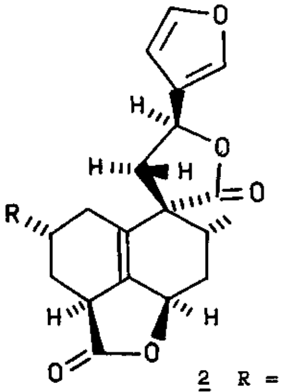
Abstract - From the aerial parts of Teucrium kotschyanum a new neoclerodane diterpenoid, isoteucrin H_4, was isolated and its structure of (12*S*)-15,16-epoxy-2α-hydroxy-19-nor-neocleroda-5(10),13(16),14-triene-18,6β;20,12-diolide (2) was established by spectroscopic means and by partial synthesis from teucrin H_4 (1). Isoteucrin H_4 is the first 19-nor-neoclerod-5(10)-ene derivative found in nature and has biogenetic importance, since structures with a C-5,C-10 double bond were postulated as intermediates in the biosynthesis of the diterpenoids belonging to the H-10α-19-nor-neoclerodane series.

From the acetone extract of the aerial parts of Teucrium kotschyanum Poech. (synonym: T. smyrnaeum Boiss.)[1], family Labiatae, we isolated[2], among other substances, the previously[3,4] known neoclerodane diterpenoid teucrin H_4 (1) and a new compound, isoteucrin H_4, whose structure (2) was established as follows.
Isoteucrin H_4 (2) was very difficult to purify and after obtaining its ^{1}H-nmr spectrum (Table 1) was purified by transformation into its acetyl derivative 3 [mp 191-194° (EtOAc-*n*-hexane); $[\alpha]_D^{22}$+128.6° ($CHCl_3$, c 0.168)]. Elemental analysis and mass spectroscopy[5] gave the molecular formula of compound 3 as $C_{21}H_{22}O_7$. Its ir spectrum was consistent with the presence of a furan ring (3140, 3120, 1505, 875 cm^{-1}), γ-lactone groups (strong and broad absorption at 1767 cm^{-1}) and acetate group (1735, 1240 cm^{-1}). Furthermore, the ^{1}H-nmr spectrum of 3 was almost identical with that of the acetyl derivative[6] (4) of teucrin H_4 (Table 1), thus suggesting closely related structures for both compounds.

Dedicated to Sir Derek H. R. Barton on the occasion of his 70th birthday.



1 R = OH
4 R = OAc



2 R = OH
3 R = OAc
5 R = H

Comparison of the ^{13}C-nmr spectra of compounds 3 and 4 (Table 2) clearly established that the new diterpenoid had a C-5,C-10 tetra-substituted double bond instead of the α,β-unsaturated C-18,C-6β γ-lactone moiety of the derivative 4. The olefinic carbon atom resonances of compound 3 at δ 132.21 and 126.17 compared with those of 4 (δ 122.53 and 165.70) firmly supported this view. In agreement with this conclusion, compound 3 showed uv absorption at 213 nm (log ε 3.86), almost identical with that reported[7] for the synthetic derivative 5 (λ_{max} 210 nm, log ε 3.96) and quite different from those of 4 (λ_{max} 223 nm, log ε 4.05)[6] and other neoclerod-4-en-18,6-olide derivatives[8].

The configurations at the C-4, C-6 and C-12 centres of isoteucrin H_4 were established by nOe experiments[9], which also confirmed the C-2α configuration of the hydroxy group of teucrin H_4 (1) and established a 12S stereochemistry of its C-12 centre[9], a structural feature of this diterpenoid not previously reported[3]. Table 3 summarizes the results of the nOe experiments and shows that the acetyl derivatives of isoteucrin H_4 and teucrin H_4 have the relative stereochemistry depicted in the formulae 3 and 4 respectively, since the nOe enhancements observed are compatible only with these structures[9].

Final proof on structure and absolute configuration 2 for isoteucrin H_4 was obtained by treating teucrin H_4 acetate (4) with a sodium carbonate THF-H_2O solution at room temp. for 6 days[7], that predominantly gave a compound identical in all respects (mp, mmp, $[\alpha]_D$, ir, uv, ^{1}H-nmr, ms and TLC) with the acetyl derivative 3. Isoteucrin H_4 (2) is the first 19-nor-neoclerodane derivative isolated from a natural source which has a C-5,C-10 double bond, and its occurrence in *Teucrium kotschyanum* supports previous hypotheses[7,10] on the biogenesis of the 19-nor-clerodane derivatives belonging to the H-10α series, like teucvidin and teuflidin[8]. Natural diterpenoids with an olefinic C-5,C-10 double bond are infrequent and only some abeo-20(10→9)-labd-5(10)-ene derivatives are known[11].

TABLE 1. ^{1}H-Nmr data of compounds 2-4 (300 MHz, $CDCl_3$, TMS as internal standard)*

	2	3	4
H-2β	4.42 m ($W_{\frac{1}{2}}$ 10 Hz)	5.38 m ($W_{\frac{1}{2}}$ 9 Hz)	5.24 m ($W_{\frac{1}{2}}$ 16 Hz)
H-3α	**	2.47 br ddd	**
H-3β	1.42 ddd	1.48 ddd	**
H-4α	3.50 br dd	3.38 br dd	-
H-6α	5.03 m ($W_{\frac{1}{2}}$ 18 Hz)	5.05 m ($W_{\frac{1}{2}}$ 19 Hz)	5.75 m ($W_{\frac{1}{2}}$ 17 Hz)
H-7β	1.62 ddd	1.65 ddd	**
H-8β	**	**	2.28 m **
H-10β	-	-	2.90 m **
H-11α	2.80 dd ++	2.79 dd	2.70 dd
H-11β	2.22 dd	2.22 dd	2.38 dd
H-12	5.60 ddd	5.58 ddd	5.34 dd
H-14	6.35 dd	6.35 dd	6.41 dd
H-15	7.48 t	7.48 t	7.47 t
H-16	7.43 m ($W_{\frac{1}{2}}$ 3 Hz)	7.44 m ($W_{\frac{1}{2}}$ 3 Hz)	7.50 m ($W_{\frac{1}{2}}$ 3 Hz)
Me-17	1.09 d	1.10 d	1.23 d
OAc	-	2.08 s	2.12 s

J (Hz). Compound 2: 2β,3β 1.8; 3α,3β 12.9; 3α,4α 5.1; 3β,4α 10.9; 6α,7β 9.7; 7α,7β 12.8; 7β,8β 3.5; 8β,17 7.2; 11α,11β 13.3; 11α,12 9.1; 11β,12 3.0; 12,16 1.3; 14,15 1.8; 14,16 0.9; 15,16 1.8.

Compound 3: 2β,3α 5.3; 2β,3β 2.2; 3α,3β 13.5; 3α,4α 4.5; 3β,4α 10.8; 6α,7β 9.3; 7α,7β 13.0; 7β,8β 3.8; 8β,17 7.1; 11α,11β 13.6; 11α,12 9.1; 11β,12 3.3; 12,16 1.1; 14,15 1.8; 14,16 0.9; 15,16 1.8.

Compound 4: 6α,7β 14.1; 8β,17 7.3; 11α,11β 13.7; 11α,12 7.2; 11β,12 10.0; 12,16 0; 14,15 1.8; 14,16 1.0; 15,16 1.8.

The H-6α proton showed significant homoallylic couplings with the H-4α and H-1 protons (compound 2: $J_{6\alpha,4\alpha}$ 2.4; $J_{6\alpha,1\alpha}$ 1.1; $J_{6\alpha,1\beta}$ 0.6 Hz) and with the H-3 and H-10β protons (compound 4: $J_{6\alpha,10\beta}$ 2.0; $J_{6\alpha,3\alpha}$ + $J_{6\alpha,3\beta}$ 0.9 Hz).

* Spectral parameters were obtained by first order approximation. All the assignments were confirmed by double resonance experiments.

** Overlapped signals.

++ Protons H-11α (pro-S) and H-11β (pro-R) were distinguished by nOe experiments (see Table 3); H-11β and Me-17 are on the same side of the plane defined by the γ-lactone ring.

TABLE 2. ^{13}C-Nmr chemical shifts of compounds 3 and 4 (75.4 MHz, $CDCl_3$, TMS as internal standard).

	3	4		3	4
C-1	29.27 t (*)	29.41 t	C-12	71.93 d	71.66 d
C-2	66.69 d	70.15 d	C-13	125.78 s (x)	124.08 s
C-3	26.54 t	25.75 t	C-14	107.75 d	107.81 d
C-4	35.27 d (+)	122.53 s	C-15	144.52 d	144.40 d
C-5	132.21 s	165.70 s	C-16	138.66 d	139.93 d
C-6	74.72 d	76.43 d	C-17	15.50 q	17.55 q
C-7	31.76 t	31.72 t	C-18	176.05 s (")	172.24 s
C-8	36.02 d (+)	36.20 d	C-20	175.94 s (")	175.57 s
C-9	50.68 s	50.78 s	O$\underline{C}$OCH$_3$	170.43 s	170.54 s
C-10	126.17 s (x)	41.22 d	OCO$\underline{C}$H$_3$	21.28 q	21.21 q
C-11	40.08 t	42.49 t			

(*) SFORD multiplicity

(+)(x)(") Assignments bearing the same sign may be interchanged, but those given here are considered to be the most likely.

TABLE 3. NOe experiments on compounds 3 and 4.

		NOe enhancements (%)							
Compound	Irradiation δ	H-4α	H-6α	H-10β	H-12	H-14	H-16	H-11α	H-11β
3	1.10 (Me-17)	0	12.5	-	0	0	1.9	0	1.5
	3.38 (H-4α)	-	6.7	-	0	0	0	0	0
4	1.23 (Me-17)	-	9.2	0	0	3.2	1.2	0	2.0
	5.24 (H-2β)	-	0	6.5	0	0	0	0	C

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REFERENCES AND NOTES

1. Plants materials were collected in July 1987, at Cedars Valley, Cyprus, 10 km east of Stavros tis Psokas, 1120 msl. Voucher specimens were deposited at the Herbarium of the Botanic Gardens of Camerino and Catania.
2. Dried aerial parts (500 g) were extracted several times with acetone at room temp.; the dried extract (36 g) was chromatographed on silica gel column (deactivated with 15% H_2O), eluent pet.ether with increasing percentages of AcOEt.

The hypothesis that isoteucrin H_4 is an extraction artifact can be ruled out because the isolation procedure is very mild. Isoteucrin H_4 and teucrin H_4 do co-occur in the plant, as TLC examination of the fresh extract shows their presence. No isoteucrin H_4 was detected in two other species of *Teucrium* containing[3,4] teucrin H_4.

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5. Isoteucrin H_4 acetate (**3**). *Anal*. Calcd for $C_{21}H_{22}O_7$: C, 65.27; H, 5.74. Found: C, 65.36; H, 5.63. Ms m/z (rel.int.): 386 $[M]^+$ (1.7), 327 (8), 326 (3), 309 (1.6), 292 (20), 274 (23), 232 (100), 204 (27), 173 (14), 162 (16), 143 (22), 129 (18), 95 (23), 94 (53), 91 (13), 81 (15), 43 (32).
6. The derivative **4**, not previously described, had mp 219-221° (EtOAc-*n*-hexane); $[\alpha]_D^{22}$ -36.5° ($CHCl_3$, c 0.271); ir γ_{max}^{KBr} cm^{-1}: 3165, 3140, 3120, 1515, 878 (furan), 1770 (γ-lactone), 1750, 1702, 1610 (α,β-unsaturated γ-lactone), 1740, 1255 (acetate); uv λ_{max}^{MeOH} 223 nm (log ε 4.05); ms m/z (rel.int.): 386 $[M]^+$ (2), 371 (1), 369 (31), 344 (30), 324 (32), 232 (38), 230 (25), 199 (17) 105 (19), 96 (100), 94 (28), 91 (17), 81 (44); ^{1}H-nmr and ^{13}C-nmr see Tables 1 and 2; M_r for $C_{21}H_{22}O_7$: 386.
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