neo-Clerodane Diterpenoids from *Teucrium alyssifolium*

G. Topcu* and C. Eriş

TUBITAK, Marmara Research Center, Department of Chemistry, P.O. Box 21, 41470, Gebze, Kocaeli, Turkey

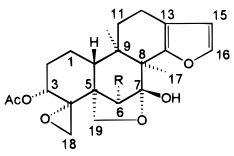
A. Ulubelen

Faculty of Pharmacy, University of Istanbul, 34452, Istanbul, Turkey, and TUBITAK, Marmara Research Center, Department of Chemistry, P.O. Box 21, 41470, Gebze, Kocaeli, Turkey

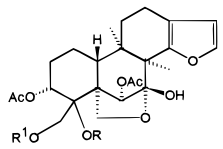
Received October 30, 1996[⊗]

Two new rearranged *neo*-clerodane diterpenoids, alysines D (**1**) and E (**2**), were isolated from the aerial parts of *Teucrium alyssifolium*. Their structures were established by spectral methods including 1D and 2D NMR techniques.

As an extension of our investigation on *Salvia* species in the family Lamiaceae,[1−5] we have recently examined *Teucrium* species[6−8] of this family in order to study chemically their diterpenoids and, when possible, their biological activities. *Teucrium* species generally contain *neo*-clerodane diterpenes in their aerial parts[9−13] and rearranged abietane diterpenes in their roots.[6,14,15] In a previous study of the aerial parts of *Teucrium alyssifolium* Stapf., we isolated four new rearranged *neo*-clerodane diterpenes with a novel skeleton, alysines A, B, and C and 3-deacetylalysine B.[7] A further investigation of the same extract has afforded two additional rearranged *neo*-clerodane diterpenes, named alysines D (**1**) and E (**2**). Although the new compounds were structurally quite similar to the previously obtained compounds (alysines A−C), the presence of a hemiketal bridge in both **1** and **2** was the main difference between these two groups of compounds. The structures of the new compounds **1** and **2** were established in the present investigation as 3α-acetoxy-4α,18-epoxy-7,19-(epoxymethylene)-6α,7β-dihydroxy-*neo*-cleroda-13,15-diene and 3α,6α-diacetoxy-4α,18-epoxy-7,19-(epoxymethylene)-7β-hydroxy-*neo*-cleroda-13,15-diene, respectively, by spectroscopic means.



1 R = OH
1a R = OAc



2 R = R[1] = H
2a R = R[1] = Ac

The HREIMS of alysine D (**1**) indicated the molecular formula $C_{22}H_{28}O_7$ (*m*/*z* 404.1834). The IR spectrum showed the presence of hydroxyl (3420 cm^{-1}) and acetyl (1740, 1240 cm^{-1}) groups, and a furan ring (3125, 1510, 880 cm^{-1}). The presence of an acetyl group was consistent with the signal at δ 169.6 in its ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum of **1** was quite similar to that of alysine A (**3**), displaying signals for furan ring protons at δ 6.10 (H-15) and 7.31 (H-16) and the signals at δ 2.78 (H-18a) and 3.14 (H-18b), which are typical of a spiro-oxirane ring situated at C-4 in *neo*-clerodane diterpenes.[16] Tertiary methyl signals were observed at δ 1.21 (Me-20) and 1.34 (Me-17), and an aliphatic acetate methyl appeared at δ 2.10. A narrow triplet at δ 4.45 (J = 2.5 Hz) was assigned to the proton geminal to an acetyl group at C-3 for the compounds bearing a spiro-oxirane ring at C-4 as in alysines A and C.[7] The chemical shift of H-3 in **1** was also consistent with an α configuration of the acetyl group at C-3. Had the acetyl group been β, H-3 would have been observed between 5.2−5.5 ppm.[17−19] The geminally coupled protons at δ 4.18 and 4.26 (J = 11 Hz) were attributed to the AB system of the epoxymethylene group at C-19. After addition of D_2O, the signals at δ 4.03 (1H, d, J = 1.5 Hz) and 4.10 (1H, s) disappeared, showing the presence of two hydroxyls, while a third signal at δ 2.95 (1H, d, J = 1.5 Hz) became a sharp singlet, indicating that the hydroxyl at δ 4.03 was secondary. The connectivity between H-10 to H_2-1 through to H-3 was confirmed by spin-decoupling experiments. When H-1α (δ 1.50) was irradiated, H-2β (2.04) collapsed to a double doublet (J = 4, 6 Hz) and H-10 (δ 2.33) to a broad singlet while H-1β (δ 1.78) was sharpened. When the latter signal was irradiated, H-1α as well as H-2α appeared as doublet of doublets of doublets (J = 6, 10, 10.5 Hz), and H-10 became a sharp doublet (J = 10.5 Hz). The irradiation of H-2α collapsed H-3 (δ 4.45) to a doublet (J = 2.5 Hz), while H-2β became a doublet of doublets of doublets (J = 2.5, 6, 10.5 Hz). The ^{13}C-NMR (APT and DEPT 135° pulse) spectra of **1** (Table 1) indicated the presence of three methyl, five methylene (for six methylene groups), five methine, and eight carbon signals, for a total of 22 carbon atoms. The quaternary carbon resonance at δ 106.6 was assigned to the hemiketal carbon, which was located at C-7 from the HETCOR and HMBC (Table 1) experiments. In the latter experiment, three-bond correlations were observed between C-7 and H_2-19, confirming the presence of an oxygen bridge between C-7 and C-19. Acetylation of **1** yielded a diacetate (**1a**) with acetyl ^{1}H-NMR signals at δ 2.06 and 2.10. The shift of the carbinol hydrogen from δ 2.95 to δ 4.30 verified that one of the hydroxyls was secondary and the other tertiary (unacetylated) and located at C-7. The placement of all the substituents was deduced from a HMBC experiment (Table 1). The ^{1}H-NMR data, as well as biogenetic considerations, supported this compound as having a secondary hydroxyl group at C-6. Stereolocation of the hydroxyl groups followed from 1D NOE and 2D NOESY experi-

* Author to whom correspondence should be addressed. Telephone: 0090 262 641 23 00. FAX: 0090 262 641 23 09. E-mail: topcu@yunus.mam.tübitak.gov.tr.

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.

S0163-3864(96)00706-9 CCC: $14.00

Table 1. ^{1}H and ^{13}C-NMR Data of Compounds **1** and **2**[a]

position	^{1}H (mult, *J* in Hz)	^{13}C	HMBC (C → H)	^{1}H (mult, *J* in Hz)	^{13}C	HMBC (C → H)
1 α	1.50 (m)	18.6		1.68 (dddd 4, 10, 10, 10.5)	18.6	
1 *β*	1.78 (m)			1.77 (ddd 4, 6, 10)		
2 α	1.52 (m)	32.9		1.58 (m)	32.9	
2 *β*	2.04 (m)			1.87 (dddd, 2.5, 6, 10, 10)	32.9	
3	4.45 (t, 2.5)	75.1	C-4, C-5	5.18 (t, 3)	75.3	C-4, C-5, C-6
4		62.3			75.1	
5		42.1			41.8	
6	2.95 (d, 1.5)	75.4		4.30 (br s)	75.4	
7		106.6			106.6	
8		51.6			51.6	
9		48.1			48.1	
10	2.33 (dd, 4, 10.5)	42.1	C-4, C-6, C-7	2.35 (dd, 3.5, 10)	41.8	C-4, C-6
11	1.40 (m)	18.6		1.44 (m)	18.6	
12	1.38 (m), 1.62 (m)	29.1		1.40 (m), 1.58 (m)	29.6	
13		115.7			115.7	
14		154.1			154.2	
15	6.10 (d, 2)	109.5	C-12, C-13	6.14 (d, 2)	109.1	C-12, C-13
16	7.31 (d, 2)	141.9		7.31 (d, 2)	141.9	
17	1.34 (s)	17.1	C-7, C-9, C-14	1.34 (s)	17.2	C-7, C-9, C-14
18	2.78 (d, 3.5), 3.14 (d, 3.5)	49.9		4.11 (d, 11), 4.53 (d, 11)	66.4	
19	4.18 (d, 11) 4.26 (d, 11)	66.7	C-7, C-5	4.24 (d, 11), 4.32 (d, 11)	66.4	C-7
20	1.21 (s)	20.2	C-8, C-10, C-11	1.15 (s)	20.2	C-8, C-10, C-11
C═O		169.6			170.6	
CH_3	2.10 (s)	21.2		2.01 (s)	21.2	
C═O					171.2	
CH_3				2.13 (s)	20.2	

[a] Recorded in $CDCl_3$, chemical shift values were reported as *δ* values (ppm) from internal standard TMS at 200 MHz NMR for proton and 50.32 MHz for carbon.

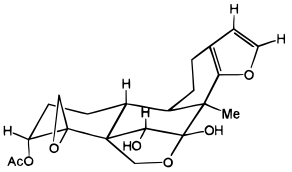


Figure 1.

ments, observing interactions between the signals at *δ* 2.33 (H-10) and 2.95 (H-6), and *δ* 4.10 (HO-7) and H-6, which indicated their *β* positions (Figure 1). All these data enabled the establishment of the structure of alysine D (**1**) as 3α-acetoxy-4α,18-epoxy-7,19-(epoxymethylene)-6α,7*β*-dihydroxy-*neo*-cleroda-13,15-diene.

The HREIMS of alysine E (**2**) indicated the molecular formula $C_{24}H_{32}O_9$ (*m*/*z* 464.2022). The ^{1}H-NMR spectrum was similar to that of compound **1**, however, in compound **2** the spiro-oxirane ring at C-4 was replaced by a CH_2OH group and an α-OH group, with the signal of the narrow triplet (H-3) at *δ* 4.45 shifted to *δ* 5.18. The carbinyl proton at *δ* 2.95 in compound **1** shifted to *δ* 4.30 (1H, s), indicating the location of the second acetyl group at C-6, with the acetyl signals observed at *δ* 2.01 and 2.13. The C-18 protons were shifted to *δ* 4.53 and 4.11, and the furan ring signals were at *δ* 6.14 (H-15) and 7.31 (H-16). Spin-decoupling experiments showed the relation between H-2*β* (*δ* 1.87) and H-2α (*δ* 1.58) and H-3*β* (*δ* 5.18) as well as between H-1α (*δ* 1.68) and H-1*β* (*δ* 1.77) and H-10 (*δ* 2.35). The unambiguous assignment of carbons and protons was made possible by HMBC and HETCOR (Table 1) experiments. In the ^{13}C-NMR (APT) spectrum of **2**, there were four methyl quartets, four methylene triplets (for six methylene groups), five methine doublets, and nine quaternary singlets for 24 carbons. Two of the quaternary signals indicated the presence of acetyl groups (*δ* 170.6 and 171.2). Acetylation of compound **2** yielded tetraacetate **2a** (see Experimental Section). Spectral data comparison to compound **1** indicated that alysine E (**2**) is 3α,6α-diacetoxy-4α,7*β*,18-trihydroxy-7,19-(epoxymethylene)-*neo*-cleroda-13,15-diene.

Experimental Section

General Experimental Procedures. The spectra were recorded with the following instruments: UV, Varian Techtron 635; IR, Perkin-Elmer 983; optical rotations, Optical Action Limited AA-5 polarimeter; ^{1}H and ^{13}C NMR, Bruker AC 200 L (200 MHz and 50.32 MHz, respectively); HMBC experiments, JEOL JNM Ex-400 MHz; HREIMS, VG ZabSpec.

Plant Material. Plant material was that used by Topcu *et al.*[7]

Extraction and Isolation. The procedure was described by Topcu *et al.*[7] The crude extract was fractionated on a Si gel column (5 × 70 cm) eluting with hexane, hexane−EtOAc gradient, and EtOAc, followed by EtOH. Compounds **1** (22 mg) and **2** (8 mg) were obtained from the column during hexane−EtOAc (7:3) elutions, and then separated from each other by preparative TLC using $CHCl_3$−Me_2CO (9:1), R_f values were 0.75 and 0.80 for compounds **1** and **2**, respectively.

Alysine D (1): amorphous compound; $[\alpha]^{25}_D$ −27.9° (*c* 0.45, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 243 (3.8) and 285 (sh) nm; IR ($CHCl_3$) ν_{max} 3420 (OH), 3125 (CH, aliphatic), 2920 (CH, olefinic), 1740 (C═O, acetyl), 1510, 1440, 1370, 1240, 1020, 880, 760 cm^{-1}; ^{1}H- and ^{13}C-NMR ($CDCl_3$, 200 and 50.32 MHz) data, see Table 1; EIMS *m*/*z* 404 $[M]^+$ (10), 345 $[M - OAc]^+$ (42), 314 $[M - OAc - CH_2OH]^+$ (10), 296 $[314 - H_2O]^+$ (12), 253 (15), 219 (20), 201 (15), 133 (50), 108 (65), 91 (45); HREIMS *m*/*z* 404.1834 (calcd for $C_{22}H_{28}O_7$, 404.1825).

Alysine E (2): amorphous compound; $[\alpha]^{25}_D$ −34.5° (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 245 (4.5) and 290 (sh) nm; IR ($CHCl_3$) ν_{max} 3560 and 3480 (OH), 3080 (CH, aliphatic), 2950, 2860 (CH, olefinic), 1745 and 1720 (C═O, acetyl), 1660, 1450, 1370, 1240, 1080, 760 cm^{-1}; ^{1}H- and ^{13}C-NMR ($CDCl_3$, 200 and 50.32 MHz) data, see Table 1; EIMS *m*/*z* 464 $[M]^+$ (12), 446 $[M - H_2O]^+$

(8), 404 [M − HOAc]$^+$ (23), 386 [404 − H_2O]$^+$ (8), 344 [M − 2 × HOAc]$^+$ (15), 256 (70), 150 (98), 108 (100), 95 (70), 79 (50); HREIMS *m/z* 464.2022 (calcd for $C_{24}H_{32}O_9$, 464.2056).

Acetylation of Compounds 1 and 2. Acetylation was carried out at room temperature for 24 h on 10 mg of **1** and 5 mg of **2** in 1 mL each of pyridine and Ac_2O. After evaporation at reduced pressure, each reaction product was purified on preparative TLC plates with $CHCl_3$−toluene (2:1) to yield of 7.8 mg **1a** and 4.2 mg of **2a**.

Acetylalysine D (1a): amorphous compound; IR ($CHCl_3$) ν_{max} 3075 (CH aliphatic), 2920 (CH olefinic), 1740 (C═O, acetyl), 1520, 1440, 1370, 1240, 760 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.21 (3H, s, Me-20), 1.34 (3H, s, Me-17), 2.06 (3H, s, OAc), 2.10 (6H, s, 2 × OAc), 2.45 (1H, d, J = 4.5 Hz, H-18a), 2.75 (1H, d, J = 4.5 Hz, H-18b), 4.22 (2H, br t, J = 11 Hz, H_2-19), 4.30 (1H, s, H-6), 4.46 (1H, t, J = 2.5 Hz, H-3β), 6.15 (1H, d, J = 2 Hz, H-15), 7.33 (1H, d, J = 2 Hz, H-16); EIMS *m/z* 446 [M]$^+$ (18), 386 [M − HOAc]$^+$ (8), 326 [386-HOAc]$^+$ (4), 311 (7), 253 (8), 161 (17), 149 (73), 83 (100).

Acetylalysine E (2a): amorphous compound; IR ($CHCl_3$) ν_{max} 3080 (CH, aliphatic), 2920 and 2860 (CH, olefinic), 1740 and 1722 (C═O, acetyls), 1515, 1450, 1380, 1240, 1030, 760 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.15 (3H, s, Me-20), 1.34 (3H, s, Me-17), 2.08, 2.10, 2.13, and 2.15 (each 3H, s, OAc), 4.22 (1H, d, J = 9 Hz, H-18a), 4.26 (1H, d, J = 10.5 Hz, H-19a), 4.30 (1H, d, J = 10.5 Hz, H-19b), 4.59 (1H, d, J = 9 Hz, H-18b), 4.32 (1H, s, H-6), 5.18 (1H, t, J = 3 Hz, H-3β), 6.20 (1H, d, J = 2 Hz, H-15), 7.38 (1H, d, J = 2 Hz, H-16); EIMS *m/z* 548 [M]$^+$ (3), 505 [M − $COCH_3$]$^+$ (8), 462 [M − 2$COCH_3$]$^+$ (20), 446 (38), 386 (25), 298 (62), 187 (75), 163 (89), 148 (100).

Acknowledgment. The authors thank Dr. Chun Tao Che, Hong Kong University of Science and Technology, for running the HMBC and 1D NOE spectra.

References and Notes

(1) Ulubelen, A.; Topcu, G.; Tan, N. *Tetrahedron Lett.* **1992**, *33*, 7241−7244.
(2) Topcu, G.; Tan, N.; Ulubelen, A.; Sun, D.; Watson, W. H. *Phytochemistry* **1995**, *40*, 501−504.
(3) Topcu, G.; Eriş, C.; Ulubelen, A. *Phytochemistry* **1996**, *41*, 1143−1147.
(4) Ulubelen, A.; Sönmez, U.; Topcu, G. Bozok-Johansson, C. *Phytochemistry* **1996**, *42*, 145−147.
(5) Topcu, G.; Ulubelen, A. *J. Nat. Prod.* **1996**, *59*, 734−737.
(6) Ulubelen, A.; Topcu, G., Ölçal, S. *Phytochemistry* **1994**, *37*, 1371−1375.
(7) Topcu, G.; Eriş, C.; Ulubelen, A.; Krawiec, M.; Watson, W. H. *Tetrahedron* **1995**, *51*, 11793−11800.
(8) Topcu, G.; Eriş, C.; Che, C.-T.; Ulubelen, A. *Phytochemistry* **1996**, *42*, 775−778.
(9) Garcia Alvarez, M. C.; Lukacz, G.; Neszmely, A.; Piozzi, F.; Rodriguez, B.; Savona, G. *J. Org. Chem.* **1983**, *48*, 5123−5126.
(10) Malakov, P. Y.; Papanov, G. Y. *Phytochemistry* **1985**, *24*, 301−303.
(11) Bruno, M.; Piozzi, F.; Savona, G.; Rodriguez, B.; de la Torre, M.; Servattaz, O. *Phytochemistry* **1987**, *26*, 2859−2861.
(12) Merritt, A. T.; Ley, S. V. *Nat. Prod. Rep.* **1992**, *9*, 243−287.
(13) Piozzi, F. *Heterocycles* **1994**, *37*, 603−626.
(14) Carreiras, M. C.; Rodriguez, B.; de la Torre, M. C.; Perales, A.; Torres, M. R.; Savona, G.; Piozzi, F. *Tetrahedron* **1990**, *46*, 847−860.
(15) Bruno, M.; de la Torre, M. C.; Savona, G.; Piozzi, F.; Rodriguez, B. *Phytochemistry* **1990**, *29*, 2710−2712.
(16) Rodriguez, B.; de la Torre, M. C.; Perales, A.; Malakov, P. Y.; Papanov, G. Y.; Simmonds, M. S. J.; Blaney, W. M. *Tetrahedron*, **1994**, *50*, 5451−5468.
(17) Al-Yahya, M. A.; Muhammet, I.; Mirza, H. H.; El-Feraly, F. S.; McPhail, T. A. *J. Nat. Prod.* **1993**, *56*, 830−842.
(18) Achari, B.; Giri, C.; Saha, C. R.; Dutta, P. K.; Pakrashi, S. C. *Phytochemistry* **1992**, *31*, 338−340.
(19) Bruno, M.; Dominguez, G.; Lourenco, A.; Piozzi, F.; Rodriguez, B.; Savona, G.; de la Torre, M.; Arnold, N. A. *Phytochemistry* **1991**, *30*, 3693−3697.

NP9607060