Neoclerodane Diterpenoids from *Teucrium chamaedrys* subsp. *syspirense*

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From the aerial parts of *Teucrium chamaedrys* ssp. *syspirense* two new neoclerodane diterpenoids, syspirensins A (**1**) and B (**2**), were isolated, together with a known phenylpropanoid triglycoside, teucrioside. The structures of **1** and **2** were established predominantly through the application of extensive ^{1}H- and ^{13}C-NMR, and 1D- and 2D-homonuclear and heteronuclear correlation NMR experiments.

Teucrium species (Labiatae) are used in traditional folk medicine, with *Teucrium chamaedrys* being one of the most highly investigated species in this genus. Preparations made from *T. chamaedrys* and *T. polium* are used as stimulants, tonics, diaphoretics, diuretics, and treatments for stomach pain. As a result of our continuing interest in the genus *Teucrium*,[2] we now report the isolation and structure elucidation of two new diterpenoids of the neoclerodane type (**1** and **2**), as well as teucrioside, a known phenylpropanoid triglycoside, from *T. chamaedrys* L. ssp. *syspirense* (C. Koch) Rech., which is one of five subspecies of *T. chamaedrys* in the flora of Turkey.[3]

Compound **1** had the molecular formula $C_{19}H_{24}O_6$ as determined by EIMS, FABMS (*m/z* 371 $[M + Na]^+$), and ^{13}C-NMR measurements. Of the eight degrees of unsaturation indicated by the molecular formula of **1**, four were present as multiple bonds (δ 133.1 s, 134.4 s, 126.9 s, 109.3 d, 145.5 d, 143.1 d, 180.0 s), indicating the molecule to be tetracyclic. In its IR spectrum there were absorbances consistent with the presence of furan (3155, 1605, 1505, 875 cm^{-1}), hydroxyl (3400 cm^{-1}), and γ-lactone (1750 cm^{-1}) functionalities. The presence of a β-substituted furan ring was also supported by the EIMS, UV, ^{1}H-, and ^{13}C-NMR data of **1**, as was the presence of a γ-lactone moiety, thus accounting for two of the four rings and three of the oxygen atoms within **1**, and indicating the remaining oxygen atoms to be present as one primary and two secondary hydroxyl functions. The EIMS of **1** contained fragment ions at *m/z* 81, 95, and 94 with relative intensities characteristic of a diterpene furanolactone.[4] In the ^{1}H-NMR spectrum of **1**, the resonances at δ 7.65, 7.58, and 6.53 were assigned to two α-furan protons and one β-furan proton, respectively. The presence of the previously mentioned γ-lactone moiety was further supported by the signals observed as an ABX system at δ 5.59 (t, J = 8.6 Hz; X part of ABX system, H-12), δ 2.51 (dd, J = 8.6 and 14.1 Hz; A part of ABX system, H-11a), and δ 2.66 (dd, J = 14.1 and 8.6 Hz; B part of ABX system, H-11b), and by the corresponding characteristic resonances in the ^{13}C-NMR spectrum.

After assigning all of the ^{1}H-NMR resonances to their respective carbon atoms via ^{1}H−^{13}C 2D shift-correlated one-bond (HMQC) measurements, it was possible to develop three other major molecular fragments from the ^{1}H−^{1}H couplings (Scheme 1): (a) starting from the methine proton assigned as H-10 (δ 3.07, br t, J = 6.5 Hz, H-10), it was possible to develop fragment 1 as consisting of a pair of methylene protons with resonances at δ 1.79 and 2.04 (H_2-1), a methine proton on an oxygen-bearing (OH) carbon atom (H-2; δ 4.08), and another pair of methylene protons assigned to H_2-3 (δ 2.17 and 2.51); (b) fragment 2 was developed beginning from the resonance of a secondary methyl at δ 1.05 (d, J = 6.8 Hz, H_3-17) to a methine (δ 2.28, m, H-8), then to a pair of methylene protons (δ 1.78 and 2.17, H_2-7), and finally to a methine proton on another oxygen-bearing (OH) carbon atom (δ 4.95, t, J = 2.9 Hz, H-6); (c) fragment 3 was composed of H_2-18 (δ 4.17 and 4.22; J_{AB} = 12.2 Hz), which was clearly an allylic hydroxymethylene function. To associate these three fragments and the furan and lactone rings it was necessary to perform a long-range ^{1}H-^{13}C (HMBC) NMR measurement. From the results of this experiment (Table 1) it was evident that the furan and the γ-lactone rings were connected via a C−C bond between C-12 and C-13, on the basis of the ^{1}H-^{13}C correlation observed between H-11 and C-13. From these results it was also clear that the lactone had to be spiro-fused at C-9 with the fragments that go to build up one of the two cyclohexane moieties within **1**. From the long-range correlations observed between H-10 and C-5, H_2-18 and C-3, and H-7 and C-9 it was also clear that the two remaining rings were both cyclohexane-based and fused so as to generate a decalin. The only bond that was not deduced from these data, and was thus inferred, is that between C-10 and C-9.

Scheme 1. Fragments of Compound **1** Deduced from 2D-NMR Measurements

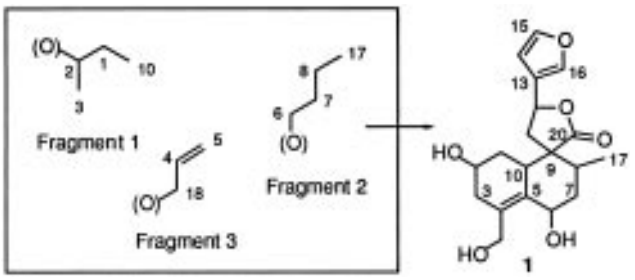

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Table 1. ^{1}H-^{13}C Heteronuclear Multiple-Bond Correlations (HMBC, J = 8.3 Hz) for Compounds **1** and **2**

carbon	HMBC for **1**	HMBC for **2**
1	H-3, H-10	H-10
2	H-1, H-3	H-10
3	H-1, H-18	H-1, H-18
4	H-3, H-18	H-2, H-10, H-18, H-19
5	H-1, H-6, H-10, H-18	H-7, H-10, H-18, H-19
6	H-7	H-7, H-19
7	H-8, H-17	H-17
8	H-7, H-11, H-17	H-20
9	H-1, H-7, H-11, H-17	H-10, H-20
10	H-1, H-11	H-2, H-20
11	H-8, H-12	H-12, H-20
12	H-11, H-14	H-14
13	H-11, H-12, H-14, H-15, H-16	H-11, H-12, H-15
14	H-12, H-15, H-16	H-12, H-15
15	H-14, H-16	
16	H-12, H-14, H-15	H-12, H-14
17	H-7, H-8	
18	H-3	
19		H-10, H-18
20	H-11	H-10, H-11

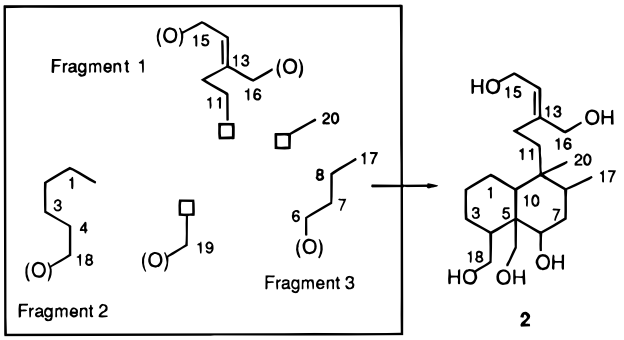
The resonances at δ 4.08 and 4.95 were conclusively assigned to H-2 and H-6, as upon acetylation the resonances for these protons were significantly deshielded in the ^{1}H-NMR spectrum of the acetoxyl derivative, **1a** (δ 5.04 and 5.97, respectively). The placement of the two secondary hydroxyl functions was further supported by the results contained in the COSY and HMQC spectra performed on **1**. From these spectra and the previous ^{1}H- and ^{13}C-NMR data it was also evident that the third hydroxyl group was primary and located at C-18. Further confirmation of this deduction once again came from the derivatization of **1** to its acetate **1a**. In **1a** the resonances for H_2-18 were significantly deshielded. Additionally, the carbon resonances at 133.1 and 134.4 ppm were ascribed to the $\Delta^{4,5}$ double bond, on the basis of HMBC correlations (Table 1).

Although the planar structure of **1** had been deduced, seven centers still required stereochemical assignment. From the ^{1}H-NMR data of **1** it was evident that both H-2 and H-6 were equatorial, inasmuch as their resonances contained no couplings greater than 3 Hz. From the NOESY spectrum of **1**, cross-peaks observed between H-10 and H-8, and between H-10 and H-3$_{ax}$, indicated these three protons to be axial. The latter interaction also indicated H-2 to be β in relation to H-3$_{ax}$, H-8, and H-10. Further NOE interactions between H-10 and H_2-11 indicated H_2-11 also to be β, whereas the cross-peak observed between H-14 and H_3-17 revealed the relative configuration at C-12 to be *S*. Thus, the structure of compound **1** was established as 15,16-epoxy-2α,6β,18-trihydroxy-19-*nor*-neocleroda-4,-13(16),14-trien-20,12(*S*)-olide, for which the trivial name syspirensin A is proposed.

Compound **2** had the molecular formula $C_{20}H_{36}O_5$ by EIMS. The ^{13}C-NMR spectrum of **2** displayed 20 carbon signals (2 × CH_3, 10 × CH_2, 5 × CH, and 3 × C). Inspection of the ^{1}H- and ^{13}C-NMR spectra of **2** indicated the presence of a 5-hydroxy-3-hydroxymethyl-3-pentenyl moiety, which is characteristic for diterpenoids found in *Portulaca* species.[5,6] The absence of resonances for any other multiple bonds indicated **2** to be a bicyclic compound.

From the HMQC and HMBC spectra of **2**, in addition to resonances for a tertiary methyl (δ 0.77, H_3-20) and a hydroxymethylene (δ 3.99 and 4.27; AB system, J_{AB} = 12 Hz) functionality, it was further possible to develop several other main molecular fragments leading to the bicyclic moiety of **2** (Scheme 2). The signals observed as an ABX system at δ 3.77 (1H, dd, J_{AB} = 11.2 Hz, J_{BX} = 4.2 Hz, H-18a), and δ 4.13 (1H, dd, J_{AB} = 11.2 Hz, J_{AX} = 7.0 Hz, H-18b) were attributed to the protons of the hydroxymethylene group located at C-4 (δ 56.1 d, C-4; δ 1.51 m, X part of ABX system, H-4). The methine proton H-4 was part of a spin network consisting of the three methylene groups, ascribed to H_2-3 (δ 1.68 and 1.63), H_2-2 (δ 1.94), and H_2-1 (δ 1.63 and 1.38), and a methine proton H-10 (δ 1.21) (fragment 2). From a 2D ^{1}H,^{1}H-homonuclear COSY spectrum of **2** it was also possible to establish the sequence $CH_3CHCH_2CH(OH)-$, corresponding to the C-17 to C-6 moiety of the B ring of the decalin moiety (fragment 3). Thus, H_3-17 (δ 0.91, d, J = 6.0 Hz) was coupled to H-8 (δ 1.74, m), which further coupled to a pair of methylene protons (δ 1.68 and 1.77, each 1H, m, H_2-7), which intercoupled and further coupled to a methine proton attached to an oxygen-bearing (OH) carbon atom (δ 3.63 dd, J = 10.0, 4.0 Hz, H-6).

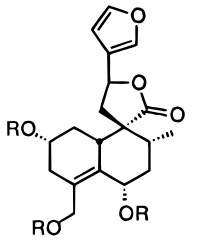
Scheme 2. Fragments of Compound **2** Deduced from 2D-NMR Measurements



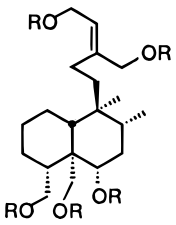
As CH_3-20 was a singlet resonance in the ^{1}H-NMR spectrum of **2** and its ^{1}H-^{13}C couplings in the HMBC spectrum were to C-8 (δ 35.8, d), C-9 (δ 39.5, s), and C-11 (δ 38.4, t), it clearly had to be positioned at C-9 together with fragment 1. A further long-range correlation observed from C-9 to H-10 (δ 1.21) established the connection of fragments 1 and 3 to 2 via C-9. The tertiary carbon atom C-5 (δ 47.4) showed HMBC correlations to H_2-7, H-10, H_2-18, and H_2-19, indicating it to be the point of closure to generate the B ring of the decalin. The remaining connection between the A and B rings was clear from the couplings of C-4 (δ 56.1, d) to H_2-18 and H_2-19, giving rise to the planar structure of **2** (see Table 1).

The relative stereochemistry of the decalin skeleton of **2** was established by 2D NOESY measurement. The spectrum that resulted from this measurement contained cross-peaks between H_3-17 and H_3-20 and H_2-19. These results indicated the H_3-17, H_3-20, and H_2-19 all to be α. NOE cross-peaks between H-4 and H-6, H-6 and H-8, H-6 and H-10, H-8 and H-10, and H-10 and H-11 revealed them all to be β. These data also revealed the decalin moiety to be trans-fused and the two cyclohexane rings to adopt chair conformations. Further, an NOE cross-peak between H-14 and H_2-16 indicated $\Delta^{13,14}$ to be *Z*. The relative stereochemistry at all centers is thus as shown in **2**. Acetylation of **2** yielded the pentaacetate **2a**, whose spectral data were all consistent with deductions made for **2**. Thus,

the structure of compound **2** was established as 6α,15,16,18,19-pentahydroxyneoclerod-13-ene, for which the trivial name syspirensin B is proposed.



1 R = H
1a R = Ac



2 R = H
2a R = Ac

The spectral data obtained for teucrioside (see Experimental Section) were identical to those published.[2]

Experimental Section

General Experimental Procedures. For the experimental procedures, see Çalis *et al.*[7]

Plant Material. *Teucrium chamaedrys* subsp. *syspirense* was collected from central Anatolia, Turkey, between Ankara and Kalecik (ca. 20 km from Kalecik) in July 1993. A voucher specimen (No. 92083) has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University.

Extraction and Isolation. Air-dried, powdered aerial parts (225 g) were extracted with MeOH at room temperature. After evaporation under vacuum, the residue was dissolved in H_2O and defatted with petroleum ether. The H_2O-soluble parts of the MeOH extract (28 g) were chromatographed on polyamide, eluting with H_2O containing increasing amounts of MeOH to give seven fractions, A−G (fractions A, 2.7 g; B, 0.95 g; C, 1.12 g; D, 1.40 g; E, 0.6 g; F, 0.3 g; G, 0.25 g). Part of fraction B (380 mg) was subjected to MPLC (column dimensions 352 × 18 mm, packed with Sepralyte 40 μM) using gradient elution, H_2O to 30−50% MeOH, to afford compounds **3** (59 mg) and **1** (13 mg), while from fraction D (950 mg), using similar conditions, compounds **3** (195 mg) and **2** (21 mg) were obtained.

Syspirensin A (1): obtained as an amorphous colorless powder; UV (MeOH) λ max 214.5 nm; IR (KBr) ν max 3400, 3155, 1750, 1605, 1505, 875 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 7.65 (1H, dd, J = 1.8, 0.8 Hz, H-15), 7.58 (1H, dd, J = 1.8, 0.8 Hz, H-16), 6.53 (1H, dd, J = 1.8, 0.8 Hz, H-14), 5.59 (1H, t, J = 8.6 Hz, H-12), 4.95 (1H, t, J = 2.9 Hz, H-6), 4.17, 4.22 (2H, AB system, J_{AB} = 12.2 Hz, H_2-18), 4.08 (1H, m, H-2), 3.07 (1H, dd ("br t"), J = 6.5 Hz, H-10), 2.66 (1H, dd, J = 14.1 and 8.6 Hz, H-11b), 2.51 (1H, dd, J = 14.1 and 8.6 Hz, H-11a), 2.51 (1H, m, H-3a), 2.28 (1H, m, H-8), 2.17 (2H, m, H-3b, H-7a), 2.04 (1H, m, H-1a), 1.79 (1H, m, H-1b), 1.78 (1H, m, H-7b), 1.05 (3H, d, J = 6.8 Hz, H_3-17); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 180.0 (s, C-20), 145.5 (d, C-15), 143.1 (d, C-16), 134.4 (s, C-5), 133.1 (s, C-4), 126.9 (s, C-13), 109.3 (d, C-14), 74.0 (d, C-12), 65.3 (d, C-6), 64.7 (d, C-2), 62.1 (t, C-18), 54.9 (s, C-9), 41.6 (t, C-11), 40.1 (d, C-10), 38.0 (t, C-7), 37.2 (t, C-3), 34.1 (d, C-8), 33.5 (t, C-1), 17.6 (q, C-17); EIMS m/z $[M - H_2O]^+$ 330 (82), $M - [2H_2O]^+$ 312 (7), 285 (20), 267 (11), 249 (34), 236 (35), 235 (27), 218 (41), 203 (20), 192 (22), 185 (27), 179 (30), 178 (41), 161 (40), 145 (45), 105 (52), 95 (100), 94 (46), 91 (74), 81 (56), 77 (46); FABMS m/z $[M + Na]^+$ 371 (calcd for $C_{19}H_{24}O_6$, 348).

Syspirensin B (2): amorphous colorless powder; UV λ max 214.5 nm; IR ν max 3435, 2925, 1635 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 5.49 (1H, t, J = 6.9 Hz, H-14), 4.27, 3.99 (2H, AB system, J_{AB} = 12.0 Hz, H_2-19), 4.16 (1H, d, J = 6.6 Hz, H-15), 4.13 (2H, s, H_2-16), 4.13 (1H, dd, J = 11.2, 7.0 Hz, H-18b), 3.77 (1H, dd, J = 11.2, 4.2 Hz, H-18a), 3.63 (1H, dd, J = 10.0, 4.0 Hz, H-6α), 2.08 (1H, m, H-12a), 1.94 (2H, m, H_2-2), 1.91 (1H, m, H-12b), 1.74 (1H, m, H-8), 1.77 (1H, m, H-7a), 1.68 (2H, m, H-7b, H-3a), 1.63 (2H, m, H-3b, H-1a), 1.51 (2H, m, H-4, H-11a), 1.49 (1H, m, H-11b), 1.38 (1H, m, H-1b), 1.21 (1H, dd, J = 12.3 and 0.6 Hz, H-10), 0.91 (3H, d, J = 6.0 Hz, H_3-17), 0.77 (3H, s, H_3-20); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 143.8 (s, C-13), 127.4 (d, C-14), 81.2 (d, C-6), 66.6 (t, C-18), 62.3 (t, C-19), 60.2 (t, C-16), 58.7 (t, C-15), 56.1 (d, C-4), 50.6 (d, C-10), 47.4 (s, C-5), 39.5 (s, C-9), 38.4 (t, C-11), 38.2 (t, C-7), 35.8 (d, C-8), 29.0 (t, C-12), 28.0 (t, C-2), 27.4 (t, C-3), 22.3 (t, C-1), 18.7 (q, C-20), 16.1 (q, C-17); FABMS m/z 379 $[M + Na]^+$, 357 $[M + H]^+$ (calcd for $C_{20}H_{36}O_5$, 356).

Teucrioside: spectral data (UV, IR, FABMS, ^1H-, and ^{13}C-NMR) identical to those reported.[1]

Acetylation of Compounds 1 and 2: Compounds **1** and **2** (each 6−8 mg) were separately dissolved in pyridine and treated with Ac_2O at room temperature overnight. After workup the peracetyl derivatives **1a** and **2a** were obtained, respectively.

Syspirensin A triacetate (1a): IR ν max 2925, 1734, 1507, 1435, 1373, 1230, 1024, 760 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.47 (dd, J = 1.8, 0.8 Hz, H-15), 7.43 (1H, dd, J = 1.8, 0.8 Hz, H-16), 6.40 (1H, dd, J = 1.8, 0.8 Hz, H-14), 5.97 (1H, t, J = 2.9 Hz, H-6), 5.55 (1H, t, J = 8.7 Hz, H-12), 5.04 (1H, m, H-2), 4.63, 4.96 (2H, AB system, J_{AB} = 12.0 Hz, H_2-18), 2.80 (1H, dd ("br t"), J = 6.5 Hz, H-10), 2.06, 2.03, and 2.01 (each 3H, s, aliphatic acetyl × 3), 1.78−2.60 (9H, H_2-1, H_2-3, H_2-7, H-8, H_2-11), 0.99 (3H, d, J = 6.6 Hz, H_3-17).

Syspirensin B pentaacetate (2a): IR ν max 2930, 1734, 1720, 1560, 1420, 1046, 773 cm^{-1}; ^1H-NMR (300 MHz, $CDCl_3$) δ 5.52 (t, J = 6.9 Hz, H-14), 4.76, 4.23 (2H, AB system, J_{AB} = 12.5 Hz, H_2-19), 4.69 (1H, dd, J = 11.0 and 4.1 Hz, H-6β), 4.64 (1H, d, J = 6.6 Hz, H-15), 4.61 (2H, s, H_2-16), 4.51 (1H, dd, J = 11.2, 3.7 Hz, H-18a), 3.97 (1H, dd, J = 11.2, 7.2 Hz, H-18b), 2.08, 2.07, 2.06 (3H each, s, aliphatic acetyl × 3), 2.00 (6H, s, aliphatic acetyl × 2), 1.93 (1H, m, H-12a), 1.90 (2H, m, H_2-2), 1.77 (2H, m, H-7a, H-12b), 1.75 (1H, m, H-3a), 1.69 (1H, m, H-8), 1.59 (1H, m, H-4β), 1.57 (1H, m, H-1a), 1.52 (1H, m, H-7b), 1.49 (1H, m, H-1b), 1.38 (2H, m, H_2-11), 1.36 (1H, m, H-3b), 1.24 (1H, br d, J = 13.0 Hz, H-10), 0.80 (3H, d, J = 6.4 Hz, H_3-17), 0.70 (3H, s, H_3-20); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 171.4, 171.3, 170.8, 170.6, 170.2 (each s, $COCH_3$), 139.8 (s, C-13), 123.0 (d, C-14), 80.4 (d, C-6), 67.7 (t, C-18), 62.3 (t, C-19), 61.5 (t, C-16), 60.3 (t, C-15), 51.6 (d, C-4), 49.8 (d, C-10), 44.4 (s, C-5), 38.3 (s, C-9), 36.4 (t, C-11), 34.3 (d, C-8), 32.6 (t, C-7), 27.9 (t, C-12), 27.2 (t, C-3), 26.5 (t, C-2), 21.6, 21.2, 21.0 (each q, $COCH_3$), 21.0 (t, C-1), 20.9, 20.8 (each q, $COCH_3$), 17.7 (q, C-20), 15.3 (q, C-17); EIMS m/z $[M - OAc]^+$ 507 (<5) and 404 (<5), 307 (5), 279 (9), 247 (11), 205 (12), 187 (36), 173 (17), 167 (22), 149 (61).

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