Journal of Natural Products
Vol. 51, No. 6, pp. 1178-1183, Nov-Dec 1988

DITERPENOIDS FROM THE ROOTS OF *SALVIA HYPARGEIA*

AYHAN ULUBELEN,*

Faculty of Pharmacy, University of Istanbul, Istanbul, Turkey

NAMIK EVREN,

Narcotics Laboratories of Gendermarie, Ankara, Turkey

ERTAN TUZLACI,

Faculty of Pharmacy

and CANDAN JOHANSSON

Faculty of Medicine, University of Marmara, Istanbul, Turkey

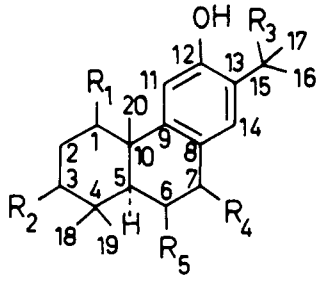
ABSTRACT.—From the root extracts of *Salvia hypargeia*, in addition to the known diterpenoids cryptanol and horminone, six new abietane diterpenoids were isolated. The structures of the new and the known compounds were established by spectral data. The new compounds, hypargenins A, B, C, D, and F, showed antibacterial activity, while hypargenin F was also active against *Mycobacterium tuberculosis*. Hypargenin E did not exhibit antibacterial activity.

In a continuation of our investigations of the diterpenoid compounds of the genus *Salvia* (1–3), we have now studied the roots of an endemic species *Salvia hypargeia* Fisch. et Mey. (Labiatae). Eight abietane diterpenoids have been isolated from this plant. Two of the compounds, cryptanol (11,12,14-trihydroxyabieta-6,8,11,13-tetraene) (1–3) and horminone (7,12-dihydroxyabieta-8,12-diene-11,14-dione) (4), were previously known diterpenoids. The other six were new compounds: hypargenin A (6β,12-dihydroxyabieta-8,11,13-triene-1,7-dione) [**1**], hypargenin B (12,15-dihydroxyabieta-8,11,13-trien-7-one) [**2**], hypargenin C (12-hydroxyabieta-8,11,13-triene-6,7-dione) [**3**], hypargenin D (12-hydroxyabieta-6,8,11,13-tetraen-3-one) [**4**], hypargenin E (6β,14-dihydroxyabieta-8,11,13-trien-1-one) [**5**], and hypargenin F (5,12-dihydroxyabieta-6,8,12-triene-11,14-dione) [**6**].

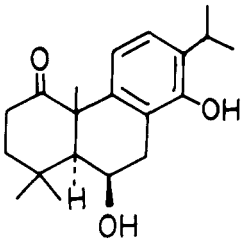
RESULTS AND DISCUSSION

The Me_2CO extract of the roots of *S. hypargeia* afforded both new and known diterpenoids. The known compounds were identified by comparing their spectroscopic data with those reported in the literature (1,4) and by comparing with authentic samples on tlc plates.

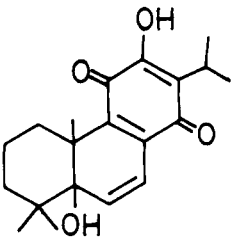
The new compounds **1–5** have spectral similarities; their ir, uv, and ^{1}H-nmr spec-



1 $R_1=R_4=O$, $R_2=R_3=H$, $R_5=\beta-OH$
2 $R_1=R_2=R_5=H$, $R_3=OH$, $R_4=O$
3 $R_1=R_2=R_3=H$, $R_4=R_5=O$
4 $R_1=R_3=R_4=R_5=H$, $R_2=O$, 6,7-dehydro



5



6

tral data indicated the presence of an aromatic ring, while those of **6** indicated a *p*-quinoid ring system. In addition, the presence of an oxo group at C-7 in compounds **1**–**3** was suggested by a peak at 1670–1680 cm^{-1} in the ir spectra of these compounds.

The molecular formula $C_{20}H_{26}O_4$ of **1** was indicated by hrms, *m/z* 330.18401. Its ir spectrum exhibited signals for aryl ketone, a six-member-ring ketone, and a hydroxyl. A uv maximum at 323 nm indicated a conjugated aromatic ring system. In the ^1H nmr of **1**, the peak at δ 7.78 (1H, s) for the C-14 aromatic proton, which is deshielded by the oxo group at C-7 as observed in similar diterpenoids cryptojaponol and sugiol (5–7), correlated with both ir and uv spectra. The downfield shift of the 20-Me signal at δ 1.7 ppm indicated the presence of a hydroxyl group at C-6 as observed in other diterpenoids with a 6-OH group (8–10). The β position of the hydroxyl group at C-6 was determined from the coupling constants of 7 Hz of H_α-5–H_α-6 pair and by studying a Dreiding model. The second oxo group must be situated in ring A, and because there are no isolated methylene group signals in the ^1H nmr spectrum, the oxo group should either be at C-1 or at C-3. In the aromatic abietane diterpenoids, H_β-1 is deshielded due to being in the plane with the aromatic ring and usually appears around 2.0–2.8 ppm (11); however, when there is a hydroxyl group at C-11, this peak moves downfield to around 3.1–3.8 ppm (9,12,13). In the ^1H-nmr spectrum of **1** there is no peak indicating H_β-1, and, therefore, the second oxo group must be at C-1 rather than at C-3 (Table 1 gives the ^1H-nmr data). The same situation was observed in compound **5**, while compound **4** showed H_β-1 at δ 2.8 indicating the presence of the second oxo group at C-3.

The high resolution mass spectrum of **2** indicated a molecular formula $C_{20}H_{28}O_3$ (*m/z* 316.20383). Its ir spectrum showed the aryl-ketone peaks. In the ^1H-nmr spectrum of **2**, the peak at δ 7.58 (1H, s, H-14) correlated with the presence of an oxo group at C-7. The presence of five methyl singlets and the lack of an isopropyl methine proton at around 3.00 ppm suggested that the second hydroxyl group should be at C-15. H_β-1 was present as a broad doublet at δ 2.8. The above data indicated that **2** is 15-hydroxysugiol.

The ^{13}C-nmr spectrum of compound **3** indicated the presence of 20 carbons, and its ^1H-nmr spectrum showed signals for 26 hydrogens. These data, in conjunction with a molecular ion at *m/z* 314 in its mass spectrum, indicated a composition $C_{20}H_{26}O_3$. The ir spectrum of **3** showed aryl-ketone peaks, as well as six-membered-ring ketone and hydroxyl peaks. The uv maximum at 403 nm together with ir peaks indicated a conjugated aromatic ring system as observed in cleons D and K (14). In the ^1H-nmr spectrum of **3** the peak at δ 7.58 (1H, s, H-14), showed the presence of an oxo group at C-7 as in compounds **1** and **2**. A 1H singlet at 2.60 (H-5) and the methyl singlet at 1.32 (20-Me), together with uv maximum, confirmed the presence of the second carbonyl at C-6 (14). Its ^{13}C-nmr spectrum showed the presence of two carbonyl groups at 191.30 ppm (C-7) (15,16) and at 202.96 ppm (C-6). Other ^1H- and ^{13}C-nmr signals were in agreement with the suggested structure (Tables 1 and 2).

TABLE 1. ^{1}H-nmr Data of Compounds **1–6**.[a]

Proton	Compound					
	1	**2**	**3**	**4**	**5**	**6**
H-1 . .	—	2.8 (1H, br d, 12)	2.8 (1H, dd, 3;12)	2.8 (1H, br d, 11)	—	2.9 (1H, br d, 12)
H-2 . .	1.4–1.6 m	1.4–2.4 m	1.0–1.8 m	1.4–2.4 m	1.5–2.5 m	1.3–2.3 m
H-3 . .				—		
H-5 . .	3.3 (1H, d, 7)	3.0 m	2.6 s	3.6 (1H, t, 3)	3.0 (1H, d, 3)	3.85 (OH)[b]
H-6 . .	3.7 (1H, d, 7)	2.4 (1H, dd, 4;12)	—	6.8 (1H, d, 9)	4.0 (1H, dt, 3;8)	6.96 (1H, d, 9)
H-7 . .	—	—	—	7.25 (1H, d, 9)	3.38 m	7.35 (1H, d, 9)
H-11 . .	6.45 s	6.47 s	6.24 s	6.9 s	7.08 (1H, d, 8)	—
H-12 . .	7.10 (OH)[b]	6.36 (OH)[b]	6.90 (OH)[b]	7.20 (OH)[b]	7.48 (1H, d, 8)	7.05 (OH)[b]
H-14 . .	7.78 s	7.58 s	7.58 s	6.92 s	6.38 (OH)[b]	—
H-15 . .	3.10[c]	5.10 (OH)[b]	3.08[c]	3.02[c]	3.10[c]	3.05[c]
H-16 . .	1.28 (3H, d, 7)	1.40 s	1.18 (3H, d, 7)	1.18 (3H, d, 7)	1.30 (3H, d, 7)	1.20 (3H, d, 7)
H-17 . .	1.32 (3H, d, 7)	1.35 s	1.20 (3H, d, 7)	1.22 (3H, d, 7)	1.34 (3H, d, 7)	1.20 (3H, d, 7)
H-18 . .	1.26 s	1.08 s	1.12 s	0.8 s	1.18 s	1.60 s
H-19 . .	1.36 s	1.10 s	1.12 s	0.8 s	1.27 s	1.60 s
H-20 . .	1.70 s	1.12 s	1.32 s	0.85 s	1.67 s	1.70 s

[a]Chemical shifts in ppm from internal TMS; coupling constants in Hz, solvent $CDCl_3$.
[b]Exchanges with D_2O.
[c]Septet, $J = 7$ Hz.

TABLE 2. ^{13}C-nmr Data of Compounds **3**, **4**, **6**, and Horminone.[a]

Carbon	Compound			
	3	**4**	**6**	horminone
C-1	29.90	29.86	30.42	33.00
C-2	26.64	28.20	27.00	25.18
C-3	33.44	207.47	32.40	36.38
C-4	39.42	39.36	34.20	46.80
C-5	43.34	41.78	83.80	42.90
C-6	202.36	130.92	127.10	25.00
C-7	191.30	139.84	126.24	69.00
C-8	126.40	127.80	124.56	124.56
C-9	140.00	141.86	142.14	147.12
C-10	42.80	42.18	41.20	40.24
C-11	126.40	127.80	185.12	185.00
C-12	146.20	156.54	153.50	152.58
C-13	140.00	144.18	128.90	125.10
C-14	145.00	144.16	184.52	184.00
C-15	22.22	23.66	22.82	25.12
C-16	21.84	22.22	21.40	23.30
C-17	21.74	21.80	21.84	22.60
C-18	21.34	21.52	21.60	19.80
C-19	21.34	21.22	21.60	18.50
C-20	18.62	21.20	20.04	18.00

[a]Solvent $CDCl_3$; chemical shifts in ppm from internal TMS.

The uv maximum of **4** at 337 nm was quite similar to that of cryptanol (332 nm) (1) indicating an aromatic ring conjugated to a double bond, which was correlated with the peaks of its ir spectrum. The ^{13}C-nmr spectrum of **4** indicated the presence of 20 carbon atoms, and its ^{1}H-nmr spectrum showed signals for 26 hydrogens. These data, together with the molecular ion peak at *m/z* 298 in its mass spectrum, indicated a molecular formula $C_{20}H_{26}O_2$. The ^{1}H-nmr spectrum showed the structure of **4** clearly, at δ 6.90 (1H, s, H-11), 6.92 (1H, s, H-14), 7.20 (1H, s, 12-OH) (D_2O exchange), 6.80 (1H, br d, J = 9 Hz, H-6), 7.25 (1H, br d, J = 9 Hz, H-7), and δ 3.6 (1H, t, J = 3 Hz, H-5). The second oxygen must be in the form of an oxo group on ring A. The observation of a broad doublet at δ 2.8 indicated the presence of H_β-1, and, therefore, the oxo group must be at C-3; it was observed at 207.47 ppm in the ^{13}C-nmr spectrum (Table 2).

The high resolution mass spectrum of **5** indicated a molecular formula $C_{20}H_{28}O_3$ (*m/z* 316.20383). The uv maximum at 274 nm suggested an aromatic ring without conjugation. The ir spectrum showed the aromatic peaks and a six-membered-ring ketone at 1715 cm^{-1}. The ^{1}H-nmr spectrum of **5** showed aromatic protons at δ 7.48 (1H, d, J = 8 Hz, H-12) and 7.08 (1H, d, J = 8 Hz, H-11) and an OH at 6.08 (1H, s, 14-OH) (D_2O exchange). A double triplet at δ 4.00 (1H, dt, J = 3 Hz and 8 Hz, H-6) and a doublet at 3.00 (1H, d, J = 3 Hz, H-5) indicated the presence of the hydroxyl group at C-6 which was correlated with the methyl singlet at δ 1.67 (3H, s, 20-Me) (8–10). The third oxygen function is a ketone and must be situated either at C-1 or at C-3 in ring A. Because there is no peak between 2.1 and 2.8 ppm for H_β-1, the oxo group is at C-1. All other peaks are in agreement with the suggested structure (Table 1). The stereochemistry at C-6 was decided by measuring the J values of H_α-6 and H_α-5 ($J_{6e,7e} = J_{6e,5a} = 3$ Hz; $J_{6e,7a} = 8$ Hz) and by studying a Dreiding model; the hydroxyl group is β.

The uv maximum of **6** at 437 nm indicated a conjugated *p*-quinoid rather than an aromatic ring system, which was consistent with the ir peaks at 1685, 1660, and 1620 cm^{-1}. The molecular ion peak at *m/z* 330 in its ms together with the presence of 20 carbon atoms as indicated in its ^{13}C-nmr spectrum and 26 hydrogen atoms in its ^{1}H nmr, as well as elemental analysis, indicated a composition of $C_{20}H_{26}O_4$. The ^{1}H-nmr spectrum suggested the isopropyl group at δ 1.2 (6H, d, $J=7$ Hz) and 3.05 (1H, septet, $J=7$ Hz). The lack of any signals between 3.05 and 6.96 ppm, together with the downfield shifts of the C-18, C-19, and C-20 methyl signals, indicated that the second hydroxyl must be at C-5. The ^{13}C nmr showed the *p*-quinoid peaks at 185.12 and 184.52 ppm for C-11 and C-14. Other peaks were in agreement with the proposed structure (Table 2). The spectral data suggested that **6** is 5-hydroxy-6,7-dehydroroyleanone (8).

The new compounds were tested against bacterial systems *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Candida albicans* as well as *Mycobacterium tuberculosis*. Hypargenins A, B, C, and F showed activity against *S. aureus*. In addition, hypargenin F was active against *S. epidermidis, Ps. aeriginosa,* and *M. tuberculosis*. Hypargenins A and B were also active against *Kl. pneumoniae* and hypargenins C and D against *B. subtilis* (Table 3).

TABLE 3. Antibacterial Activity of Hypargenins A, B, C, D, and F (MIC values μg/ml).

Compound	Bacteria				
	Staphylococcus aureus (ATCC 6538)	*Staphylococcus epidermidis* (ATCC 12228)	*Pseudomonas aeruginosa* (ATCC 1539)	*Bacillus subtilis* (ATCC 6633)	*Klebsiella pneumoniae* (UC 57)
Hypargenin A	15.6	—	—	—	15.6
Hypargenin B	125.0	—	—	—	125.0
Hypargenin C	125.0	—	—	15.6	—
Hypargenin D	—	—	—	62.5	—
Hypargenin F	125.0	62.5	125.0	—	—

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were recorded with the following instruments: ^{1}H nmr, Bruker FT 200 MHz; ^{13}C nmr, Bruker FT 50.323 MHz; ir, Perkin-Elmer 577; uv, Varian Techtron 635; ms, Finnigan-MAT 1020.

PLANT MATERIAL.—The roots of *S. hypargeia* were collected from eastern Turkey (Sivas) in July 1986; it was identified by one of us (E. Tuzlacı). A voucher specimen, MARE 479, is deposited in the Herbarium of the Faculty of Pharmacy, University of Marmara.

ISOLATION OF THE COMPOUNDS.—Air-dried and powdered roots (200 g) of the plant were extracted with Me_2CO and EtOH, respectively. The solvents were removed in vacuo. The Me_2CO concentrate (9.5 g) was separated on a Si gel column (3 × 60 cm); the diterpenoids obtained from the column were further separated and cleaned on preparative tlc plates (E. Merck). The yields were as follows: Hypargenin A (14 mg), hypargenin B (14 mg), hypargenin C (200 mg), hypargenin D (10 mg), cryptanol (4.3 mg), hypargenin E (4.2 mg), horminone (7 mg), and hypargenin F (110 mg). The EtOH extract contained flavonoids and triterpenoids and was not studied.

ANTIBACTERIAL ACTIVITY.—The disc-diffusion method (17,18) was used to measure the antibacterial activity of the new compounds. Compounds that had inhibition zones larger than 7 mm were selected for the tube dilution test (19). The lowest concentration of the sample required to inhibit the growth of the test organisms was designated as the MIC. The results are given in Table 3. All samples were tested in Löwenstein-Jensen medium (20) using *M. tuberculosis* (H 37 RV) for testing antituberculous activity: only hypargenin F showed activity at 250 μg/ml doses. The test organisms, except *M. tuberculosis*, are deposited in the Microbiology Department of the Faculty of Pharmacy, University of Istanbul; *M. tuberculosis* is deposited in the Refik Saydam Institute, Ministry of Health, Ankara, Turkey.

Hypargenin A [**1**].—Dark yellow, amorphous compound; uv λ max (MeOH) 323 (log ε 3.5), 250 (log

ε 4.2), 222 (log ε 3.7) nm; ir ν max ($CHCl_3$) 3420, 3020, 3010, 2960, 2930, 1720, 1690, 1630, 1610, 1560, 1550, 1475, 1440, 1360, 1330, 1280, 1160, 1000, 990, 850, 780 cm^{-1}; ^1H nmr see Table 1; ms (probe) 70 eV *m/z* (rel. int.) $[M]^+$ ($C_{20}H_{26}O_4$) 330 (1), $[M-16]^+$ 314 (5), $[M-16-15]^+$ 299 (5), 244 (10), 229 (5).

Hypargenin B [**2**].—Orange, amorphous compound; uv λ max (MeOH) 310 (log ε 3.5), 252 (log ε 4.1), 221 (log ε 3.7); ir ν max ($CHCl_3$) 3420, 3030, 2960, 2930, 2870, 1680, 1630, 1615, 1580, 1520, 1460, 1430, 1400, 1360, 1295, 1250, 1170, 1140, 1120, 1080, 1060, 1050, 970, 750 cm^{-1}; ^1H nmr see Table 1; ms (probe) 70 eV *m/z* (rel. int.) $[M]^+$ ($C_{20}H_{28}O_3$) 316 (3), $[M-18]^+$ 298 (5), 287 (5), 281 (10), 243 (5), 229 (20).

Hypargenin C [**3**].—Orange, amorphous compound; uv λ max (MeOH) 403 (log ε 3.7), 333 (log ε 4.2), 320 (log ε 4.1), 218 (log ε 3.5); ir ν max ($CHCl_3$) 3420, 3030, 2960, 2930, 2870, 1720, 1670, 1637, 1620, 1610, 1590, 1550, 1510, 1460, 1420, 1390, 1350, 1290, 1140, 1050, 990, 900, 810 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; ms (probe) 70 eV *m/z* (rel. int.) $[M]^+$ ($C_{20}H_{26}O_3$) 314 (50), $[M-15]^+$ 299 (10), $[M-28]^+$ 286 (38), $[M-28-15]^+$ 271 (40), 245 (28), 231 (25), 201 (20), 187 (20).

Hypargenin D [**4**].—Orange, amorphous compound; uv λ max (MeOH) 337 (log ε 3.6), 246 (log ε 4.5), 224 (log ε 4.3) nm; ir ν max ($CHCl_3$) 3450, 3060, 2960, 2930, 2870, 1730, 1635, 1595, 1580, 1505, 1460, 1395, 1370, 1260, 1230, 1205, 1180, 1100, 1030, 980, 910, 800, 710 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; ms (probe) 70 eV *m/z* (rel. int.) $[M]^+$ ($C_{20}H_{26}O_2$) 298 (18), 229 (100), 201 (20), 185 (15).

Hypargenin E [**5**].—Colorless compound; uv λ max (MeOH) 274 (log ε 3.6), 240 (log ε 3.8) nm; ir ν max ($CHCl_3$) 3500, 3045, 2960, 2850, 1715, 1600, 1565, 1500, 1450, 1410, 1370, 1310, 1220, 1170, 1110, 1000, 940, 880, 780 cm^{-1}; ^1H nmr see Table 1; ms (probe) 70 eV *m/z* (rel. int.) $[M]^+$ ($C_{20}H_{28}O_3$) 316 (12), 269 (2), 255 (3), 229 (50), 201 (5).

Hypargenin F [**6**].—Dark red compound; uv λ max (MeOH) 437 (log ε 4.2), 343 (log ε 3.8), 260 (log ε 4.5), 220 (log ε 4.3) nm; ir ν max ($CHCl_3$) 3480, 3320, 2960, 2930, 2870, 1685, 1660, 1620, 1580, 1565, 1495, 1460, 1410, 1380, 1250, 1170, 940, 820 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; ms (probe) 70 eV *m/z* (rel. int.) $[M]^+$ ($C_{20}H_{26}O_4$) 330 (10), $[M-18]^+$ 312 (15), $[M-2\times18]^+$ 294 (35), 281 (35), 243 (30), 227 (60). Found C 72.75, H 7.90; $C_{20}H_{26}O_4$ requires C 72.72, H 7.87%.

LITERATURE CITED

1. A. Ulubelen, G. Topcu, and B. Terem, *Phytochemistry*, **26**, 1534 (1987).
2. G. Topcu, A. Ulubelen, and B. Terem, *Fitoterapia*, **58**, 281 (1987).
3. A. Ulubelen and E. Tuzlacı, *Planta Med.*, **53**, 578 (1987).
4. K.S. Mukherjee, P.K. Ghosh, and S. Badruddoza, *Phytochemistry*, **20**, 1441 (1981).
5. E. Wenkert, J. de P. Campello, J.D. McChesney, and D.J. Watts, *Phytochemistry*, **13**, 2545 (1974).
6. R.C. Cambie, R.J. Madden, and J.C. Parnell, *Aust. J. Chem.*, **24**, 217 (1971).
7. S.B. Katti, P. Rüedi, and E.U. Conrad, *Helv. Chim. Acta*, **65**, 2189 (1982).
8. M. Hensh, P. Rüedi, and C.H. Eugster, *Helv. Chim. Acta*, **58**, 1921 (1975).
9. T. Miyase, P. Rüedi, and C.H. Eugster, *Helv. Chim. Acta*, **60**, 2770 (1977).
10. M. Moir, P. Rüedi, and C.H. Eugster, *Helv. Chim. Acta*, **56**, 2539 (1973).
11. L.J. Harrison and Y. Asakawa, *Phytochemistry*, **26**, 1211 (1986).
12. R.V. Stevens and G.S. Bisacchi, *J. Org. Chem.*, **47**, 2396 (1982).
13. J.A. Hueso-Rodriguez, M.L. Jimeno, B. Rodriguez, G. Savona, and M. Bruno, *Phytochemistry*, **22**, 2005 (1983).
14. P. Rüedi and C.H. Eugster, *Helv. Chim. Acta*, **60**, 1233 (1977).
15. J. Escudera, L. Perez, R.M. Rabanal, and S. Valverde, *Phytochemistry*, **22**, 585 (1983).
16. B.M. Fraga, A.G. Gonzales, J.R. Herrara, J.G. Lois, and A.G. Ravelo, *Phytochemistry*, **25**, 269 (1986).
17. J.D. Sleigh and M.C. Timburg, "Notes on Medical Bacteriology," Churchill Livingston, London, 1981, p. 43.
18. J.G. Colee, "Applied Microbiology," Blackwell Science Publications, London, 1976, p. 93.
19. F. Kavanagh, "Analytical Microbiology," Academic Press, New York, 1963, p. 125.
20. E.T. Çetin, "General and Practical Microbiology," Sermet Press, Istanbul, 1973, p. 441.

Received 18 April 1988