Pergamon

0031-9422(95)00888-8

Phytochemistry, Vol. 42, No. 1, pp. 145–147, 1996

0031-9422/96 $15.00 + 0.00

AN ABIETANE DITERPENE AND TWO PHENOLICS FROM *SALVIA FORSKAHLEI*

AYHAN ULUBELEN,*† UFUK SÖNMEZ,* GÜLAÇTI TOPCU† and CANDAN BOZOK JOHANSSON‡

*Faculty of Pharmacy, University of Istanbul 34452, Istanbul, Turkey; †TUBITAK, Marmara Research Center, Institute for Basic Sciences, Department of Chemistry, P.O. Box 21, 41470, Gebze-Kocaeli, Turkey; ‡Faculty of Pharmacy, University of Marmara, Haydarpaşa, Istanbul, Turkey

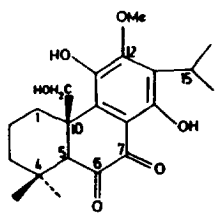
(*Received in revised form* 12 *October* 1995)

Key Word Index—*Salvia forskahlei*; Lamiaceae; forskalinone; octanol esters of *cis*- and *trans*-4-*O*-methyl-caffeic acid dimers; antimicrobial activity.

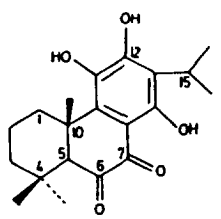
Abstract—From the roots of *Salvia forskahlei* a new diterpenoid, forskalinone, two new aromatic compounds, the octanol esters of *cis*- and *trans*-4-*O*-methyl-caffeic acid dimers, were isolated together with the known compounds stigmast-3-one, sitosterol and α-amyrin. The structures of the new and the known compounds were established by spectral data. The antimicrobial activity of forskalinone and the dimeric cinnamic acid esters was tested against standard bacterial strains and a yeast, namely *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* 6538P, *S. epidermidis* ATCC 12228, *Proteus mirabilis* ATCC 14153, *Escherichia coli* ATCC 8739, *Klebsiella pneumonia* ATC 4352, *Pseudomonus aeruginosa* ATCC 9027, *Enterococcus faecalis* ATCC 29212, *β-haemolytic Streptococcus* and *Candida albicans* ATCC 10231. Forskalinone showed moderate resistance against *S. epidermidis* (670 μg ml^{-1}) and slight activity against *E. faecalis* (168 μg ml^{-1}). *trans*-4-*O*-Methyl-caffeic acid dimer octanol ester was inactive while the *cis* isomer showed a slight activity against *C. albicans* (156 μg ml^{-1}).

INTRODUCTION

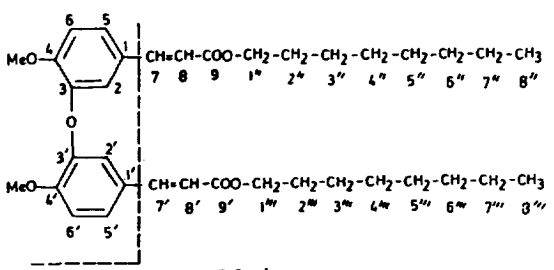
In continuation of our investigations of *Salvia* species [1–4], we have now studied the roots of *S. forskahlei* L. syn. *S. hierosolymitana* Boiss. var. *pontica* Freyn and Bornm. which has not been studied chemically and pharmacologically until now. The acetone extract of the roots yielded a new diterpenoid, forskalinone (**1**), and two new aromatic compounds, the octanol esters of *cis* (**3**) and *trans* (**4**) dimeric cinnamic acids, in addition to the known compounds stigmast-3-one, sitosterol and α-amyrin. The structures were established by spectral data. The antimicrobial activities of forskalinone and the octanol esters of *cis* and *trans* dimeric cinnamic acids were assayed against bacteria and a fungus.



1



2



3 7,8-cis 7′,8′-cis

4 7,8-trans 7′,8′-trans

RESULTS AND DISCUSSION

The new diterpene forskalinone (**1**) has the molecular formula $C_{21}H_{28}O_6$ (HREI mass spectrum, *m/z* 376.1880, calc. 376.1885). Its UV spectrum showed a long conjugation at 369 and 400 (sh) nm. The IR spectrm indicated an *ortho*-quinoid structure (1680, 1650 and 1622 cm^{-1}). The signals of the ^{1}H NMR spectrum were consistent with the structure **1**; a sharp singlet at δ 13.40 indicated a hydrogen bond between a 12). The C-11 hydroxyl group was correlated with the

diterpenoids [8, 9]. Two proton singlets of a hydroxymethylene group were seen at δ 3.64 and 3.60, which could be placed either at C-4 or C-10. When it is situated at C-4, Me-20 should be shifted upfield to δ 0.80–0.85 [10], while at C-10 the two methyl groups would have a chemical shift difference of 0.1–0.2 ppm [11] as observed in compound **1**. Other ^{1}H NMR signals were observed at δ 3.78 (3H, *s*, OMe), 3.30 (1H, *septet*, $J = 7$ Hz, H-15), 1.38 (3H, *d*, $J = 7$ Hz), 1.36 (3H, *d*, $J = 7$ Hz) (Me-16 and Me-17) and 1.34 (6H, *s*, Me-18 and Me-19). The signal at δ 2.61 (1H, *s*, H-5) indicated the presence of the second carbonyl group at C-6 and thus secured the *ortho*-quinoid structure. The ^{13}C NMR (APT) spectrum of **1** showed the presence of four methyl quartets for five methyl groups at δ 20.3, 21.5, 32.8 and 62.0 (OMe), four methylene triplets at δ 19.0, 35.8, 41.1 and 70.5 (CH_2OH), two methine doublets at δ 26.0 and 49.6 as well as 10 quaternary carbon atoms, eight of them being in the lower field at δ 184.2 and 200.6 (C-6 and C-7 carbonyls), 161.0, 153.2, 152.1, 144.6, 138.0 and 134.1 and two in the upper field at δ 40.1 and 36.4. The connectivity between the protons of C-1, C-2 and C-3 was confirmed by a ^{1}H–^{1}H COSY experiment. The spectral data indicated structure **1** for foskalinone. Cleon V (**2**), which was obtained from the Labiatae plant *Plectranthus myrianthus* BRIQ [5], has a structure which is quite similar to that of **1** apart from having a CH_2OH group instead of Me-20 and a OME group at C-12. In the ^{1}H NMR spectra of **1** and **2**, the signal of the hydrogen bond between H-14 and the C-7 oxo group is similar in both compounds. Other ^{1}H and ^{13}C NMR chemical shifts were also very similar, thus verifying the structure of **1**.

Compounds **3** and **4** were obtained together and could be separated by preparative TLC. Compound **3** is the octanol ester of *cis*-4-*O*-methyl-caffeic acid dimer with a molecular formula $C_{36}H_{50}O_7$ (*m/z* 594.3550, calc. 594.3556). The UV spectrum of **3** showed a conjugated aromatic system at 325 nm. The IR spectrum indicated an ester (1705 and 1275 cm^{-1}) and aromatic (1605, 1585 and 1520 cm^{-1}) groups. The ^{1}H NMR spectrum of **3** correlated with the suggested structure: δ 6.78 (2H, *d*, $J = 12$ Hz) (H-8 and H-8′), 5.82 (2H, *d*, $J = 12$ Hz) (H-7 and H-7′), 7.77 (2H, *d*, $J = 2$ Hz) (H-2 and H-2′), 7.10 (2H, *dd*, $J = 2$ and 8 Hz) (H-5 and H-5′), 6.87 (2H, *d*, $J = 8$ Hz) (H-6 and H-6′), 4.10 (4H, *t*, $J = 7$ Hz) (CH_2-1″ and CH_2-1‴), 3.92 (6H, *s*) (2 × OMe at C-4 and C-4′), 1.68 (4H, *m*, 2 × CH_2), 1.35 (4H, *m*, 2 × CH_2), 1.28 (12H, *br s*, 6 × CH_2), 1.15 (4H, *m*, 2 × CH_2), 0.87 (6H, *t*, $J =$ 7 Hz) (Me-8″ and Me-8‴). The ^{13}C NMR (APT) spectrum supported the suggested structure, i.e. the signals at δ 14.1 (*q*) for two methyl groups, and at δ 55.9 (*q*) for two OMe groups. The signals for methylene triplets were at δ_c 64.6 (CH_2-1″ and CH_2-1‴), 31.9 (2 × CH_2), 29.5 (4 × CH_2), 28.7 (2 × CH_2), 25.9 (2 × C-6′). The quaternary carbon signals were at δ 147.5 (C-4, C-4′), 152.3 (C-3, C-3′) and 129.5 (C-1, C-1′) and ester carbonyls were represented by one signal at δ 168.4 (C-9, C-9′). The mass spectrum of **3** contained ions at *m/z* 564 $[M - OMe]^+$, 534 $[M - 2 \times OMe]^+$, 506 $[534\text{-}CO]^+$, 478 $[506\text{-}CO]^+$ and 228 (a, $C_{14}H_{12}O_3$) and is consistent with the given structure.

Compound **4** is the *trans* isomer of compound **3**. The UV and IR spectra of both compounds are similar. In the ^{1}H NMR spectrum of **4**, the signals for the caffeic acid part showed differences at δ 7.61 (2H, *d*, $J =$ 16 Hz) (H-8, H-8′), 6.29 (2H, *d*, $J = 16$ Hz) (H-7, H-7′), 6.92 (2H, *d*, $J = 8$ Hz) (H-6, H-6′), 6.88 (2H, *dd*, $J = 2.5$ and 8 Hz) (H-5, H-5′), and 4.18 (4H, *t*, $J = 7$ Hz) CH_2-1″ and CH_2-1‴), which are consistent with *trans*-4-*O*-methyl-caffeic acid; other ^{1}H NMR signals and the mass peaks were the same in both compounds.

EXPERIMENTAL

Plant material. The roots of *S. forskahlei* were collected from Istanbul (Beykoz-Dereseki) at an altitude 300–400 m, in July 1994. The plant was identified by Dr Kerim Alpinar (Istanbul). A voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul, ISTE 66291.

Extraction and isolation of the compounds. Dried and powdered roots of the plant (1.0 kg) were extracted with distilled Me_2CO at room temp., filtered and evapd to dryness *in vacuo*, to give 24.6 g crude residue. The residue was fractionated on a silica gel column (4 × 60 cm) using a gradient of petrol, EtOAc and EtOH as eluting solvents. The frs after analyt. TLC were combined and further sepd by VLC and when necessary further on purified by prep. TLC. The following compounds were isolated: **1**, 7 mg; **3**, 12 mg; **4**, 15 mg; sitosterol, 6 mg; stigmast-3-one, 5 mg; α-amyrine, 7 mg.

Forskalinone (**1**). UV λ_{max}^{MeOH} (log ε) nm: 400 (sh), 369 (3.0), 278 (3.6), 240 (3.8). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 2925, 2870, 1680, 1650, 1622, 1455, 1415, 1377, 1250, 1175, 1100, 1060. ^{1}H NMR ($CDCl_3$): see text; ^{13}C NMR ($CDCl_3$): C-1 35.8, C-2 19.0, C-3 41.1, C-4 36.4, C-5 49.6, C-6 and C-7 184.2 and 200.6, C-8, C-9 and C-13 134.1, 138.0 and 144.6, C-10 40.1, C-11, C-12 and C-14 161.0, 153.2 and 152.1, C-15 26.0, C-16 and C-17 21.5, C-18 32.8, C-19 20.3, C-20 70.5, OMe 62.0; HREIMS *m/z* (rel. int.): 376.1880 $[M]^+$ $C_{21}H_{28}O_6$ (1), 346 $[M - CH_2OH - H]^+$ (100), 331 $[346\text{-}Me]^+$ (73), 300 $[331\text{-}OMe]^+$ (7), 261 (35), 235 (18), 207 (17), 83 (10), 69 (23).

Octanol ester of cis-O-*methyl-caffeic acid dimer* (**3**). UV λ_{max}^{MeOH} (log ε) nm: 325 (3.2), 293 (sh), 234 (4.2); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2940, 2840, 1705, 1630, 1605, 1585, 1520, 1460, 1425, 1380, 1275, 1220, 1180, 1040.

$[C_{14}H_{12}O_3]^+$ (5), 199 $[C_{14}H_{12}O_3 - OMe]^+$ (100), 153 (87), 100 (35), 85 (46), 73 (62).

Octanol ester of trans-O-*methyl-caffeic acid dimer* (**4**). UV and IR spectral data as well as HREIMS were similar to those of **3**.

Antimicrobial activity. Solns of **1**, **3** and **4** were tested against the following microorganisms: *B. subtilis*, *S. aureus*, *S. epidermis*, *P. mirabilis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E. faecalis*, a clinical isolate of *β-haemolytic Streptococcus* and *C. albicans* by a modified agar dilution method [12]. The plates contained Mueller–Hinton agar, blood agar (for *E. faecalis* and *β-haem. Streptococcus*) and Sabouraud dextrose agar media for bacteria and the yeast, respectively. They seeded with the corresponding microorganisms and the solns of **1**, **3** and **4** were dropped (50 μl) on to the media. The solvents were also tested as control. After overnight incubation, the diameters of the zones of inhibition were measured and the samples producing 5 mm inhibition zones were tested qualitatively (NCCLS broth dilution tests). The results showed that **1** was moderately active against *S. epidermidis* (670 $\mu g\,ml^{-1}$) and slightly active against *E. faecalis* (168 $\mu g\,ml^{-1}$). Compound **3** showed a slight activity against *C. albicans* (156 $\mu g\,ml^{-1}$) while **4** showed no activity.

Acknowledgement—This work was partly supported by the Research Fund of the University of Istanbul, Project No. Ö-9.

REFERENCES

1. Ulubelen, A., Topcu, G. and Tan, N. (1992) *Phytochemistry* **31**, 3637.
2. Ulubelen, A. and Topcu, G. (1992) *Phytochemistry* **31**, 3949.
3. Ulubelen, A., Topcu, G., Eriş, C., Sönmez, U., Kartal, M., Kurucu, S. and Bozok-Johansson, C. (1994) *Phytochemistry* **36**, 971.
4. Topcu, G., Ulubelen, A. and Eriş, C. (1994) *Phytochemistry* **36**, 743.
5. Miyase, T., Rüedi, P. and Eugster, C.H. (1977) *Helv. Chim. Acta* **60**, 2770.
6. Canigueral, S., Iglesias, J., Sanchez-Ferrando, F. and Virgili, A. (1988) *Phytochemistry* **27**, 221.
7. Mendes, E., Marco, J. L., Rodriguez, B., Jimeno, M. L., Lobo, A. M. and Praphakar, S. (1989) *Phytochemistry* **28**, 1685.
8. Stevens, R. V. and Bisacchi, G. S. (1982) *J. Org. Chem.* **47**, 2396.
9. Hueso-Rodriguez, J. A., Jimeno, M. L., Rodriguez, B., Savona, G. and Bruno, M. (1983) *Phytochemistry* **22**, 2005.
10. Gao, F., Leidig, M. and Mabry, T. J. (1986) *Phytochemistry* **25**, 1371.
11. Ulubelen, A. and Topcu, G. (1991) *Phytochemistry* **30**, 2085.
12. National Committee of Clinical Laboratory Standards (1990) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard. Villanova, PA.