Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 64 (2003) 395–399
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Review

Cardioactive and antibacterial terpenoids from some *Salvia* species

Ayhan Ulubelen*

Faculty of Pharmacy, Istanbul University, 34119 Istanbul, Turkey

Received 7 January 2003; received in revised form 5 March 2003; accepted 17 March 2003

Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

Seven *Salvia* species were investigated recently for their chemical and biological activities. Some terpenoidal compounds exhibited cardiovascular and antibacterial activities. A number of new diterpenoids were obtained and their structures were established through extensive spectral analysis.

Keywords: *Salvia*; Cardioactive; Antibacterial; Diterpenes

Contents

1. Introduction

Salvia species are used as traditional medicines all around the world, possessing antibacterial (Lin et al., 1989), antioxidant (Dobrynin et al., 1976), antidiabetic (Hitokato et al., 1980) and antitumor (Ulubelen et al., 1992) properties. From over 40 Turkish *Salvia* species investigated by our group we have isolated antibacterial (Miski et al., 1983; Ulubelen et al., 1988; 1994; 1996), antitumor (Topcu et al., 1997), and antituberculous (Ulubelen et al., 1997) compounds. As part of a Turkish–German project (Tubitak–Julich), seven *Salvia* species from Turkey were investigated. Cardiovascular activity was established in three of the species and antibacterial activity in four.

2. Cardioactive compounds

Salvia syriaca L. (Ulubelen et al., 2000a) yielded five diterpenoids ferruginol (**1**), 4-dehydrosalvilimbinol (**2**), viridone (**3**), candidissiol (**4**), and a new compound

* Tel.: +90-212-514-03-55; fax: +90-212-519-08.
E-mail address: aulubelen@yahoo.com (A. Ulubelen).

0031-9422/03/$ - see front matter © 2003 Published by Elsevier Ltd.
doi:10.1016/S0031-9422(03)00225-5

Table 1
Cardiovascular activities of the extracts **I**, **II,** and **III** and the compounds **1**, **7**, **14**, **15**, **19**, **20**, **22**, and **25**

Compounds	Cont. group blood press (mmHg)	Heart rate (bp m)	Exp group mean blood press (mmHg)	Heart rate (bp m)	P[a]
Crude ext. **I** ([b]$n=10$)	154.0±11.35	371.3±14.0	128±10.5	377.4±11.4	$P<0.001$
1 ($n=6$)	145.0±8.16	332.5±18.6	114±8.85	339.3±13.13	$P<0.01$
7 ($n=5$)	139.0±11.5	328.0±16.3	113±7.48	343.0±15.35	$P<0.001$
Crude ext. **II** ($n=10$)	149.0±5.7	376.0±6.8	98.0±3.8	395.0±8.2	$P<0.001$
1 ($n=10$)	133.0±3.8	395.0±3.1	110.0±5.4	397.0±4.3	$P<0.01$
14 ($n=10$)	140±5.7	375.0±8.2	105.0±3.8	395.0±5.3	$P<0.01$
15 ($n=10$)	150.0±3.9	368.0±4.5	125.0±2.8	378.0±5.7	$P<0.01$
Crude ext. **III** ($n=6$)	122.0±5.2	369.0±7.3	91.0±9.30	375.0±11	$P<0.01$
1 ($n=5$)	145.0±8.1	332.0±18.6	114.0±8.85	339.0±13.1	$P<0.01$
19 ($n=5$)	118.0±4.8	339.0±5.7	85.0±4.3	385.0±11.7	$P<0.01$
20 ($n=5$)	137.0±5.9	325.0±12.2	125.0±4.5	345.0±10.1	$P<0.01$
22 ($n=5$)	139.0±3.7	342.0±11.7	100.0±2.8	359.0±11.2	$P<0.01$
25 ($n=5$)	130.0±8.1	345.0±3.7	98.0±3.1	349.0±9.3	$P<0.01$
Propanonol[c]	153.0±33.5	379.0±43.4	93.2±11.4	319.0±29.7	$P<0.05$
Phentolamine[c]	125.0±4.1	327.0±15.2	115.0±5.7	319.0±13.4	$P<0.01$

[a] P = compound with the control.
[b] n = number of rats for test and control.
[c] Because of the widespread usage of propanonol (β-blocker) and phentolamine (α-blocker) in clinical studies, they were used as positive controls (Williams, 1998; Schüssler et al., 1995). Data are presented as mean±S.E.M. Control and experimental groups of animals were compared using Student's t-tests.

salvisyrianone (**5**), together with sitosterol (**6**), 3β-hydroxystigmast-5-en-7-one (**7**), ursolic acid (**8**), oleanolic acid (**9**), and a flavone salvigenin (**10**). The structures of the compounds were established mainly by spectral data. Extensive 1D and 2D NMR techniques, including HETCOR and COLOC, were used for determining the structure of the new compound **5**. The crude plant extract, as well as compounds **1**–**10,** were tested on Wistar Albino rats in order to establish their cardiovascular activities. Both the crude extract (**I**) and two compounds, ferruginol (**1**) and 3β-hydroxystigmast-5-en-7-one (**7**), showed antihypertensive activity by significantly reducing arterial blood pressure (Table 1).

Five diterpenoids, ferruginol (**1**), horminone (**11**), 7-acetylhorminone (**12**), sugiol (**13**), and 7-oxo-abieta-9,12,14-triene (**14**), and the steroids stigmast-4-en-3-one (**15**), stigmasterol (**16**), β-sitosterol (**6**), α-amyrin (**17**), ursolic acid (**8**), oleanolic acid (**9**), acetyloleanolic acid (**18**), and salvigenin (**10**) were isolated from *Salvia amplexicaulis* Lam. (Kolak et al., 2001). As seen in Table 1, 7-oxo-abieta-9,12,14-triene (**14**), ferruginol (**1**), stigmast-4-en-3-one (**15**), as well as the crude extract (**II**) showed a vasodepressor effect. Ulubelen et al. (2002) isolated one new, and ten known, diterpenoids from the roots of *S. eriophora* Boiss and Kotschy.The new compound was 4,14-dihydroxysaprorthoquinone (**19**), the others were aethiopinone (**20**), salvipisone (**21**), 4,12-dihydroxysapriparaquinone (**22**), 12-hydroxysapriparaquinone (**23**), 3,12-dihydroxysapriparaquinone-1-ene (**24**), 6,7-dehydroroyleanone (**25**), salvilimbinol (**26**), ferruginol (**1**), horminone (**11**), and 7-acetylhorminone (**12**). The compounds and the crude extract (**III**) were tested on Wistar Albino rats for their cardiovascular activities. Ferruginol (**1**), aethiopinone (**20**), 4,12-dihydrosapriparaquinone (**22**), 6,7-dehydroroyleanone (**25**) and 4,14-dihydroxysaprorthoquinone (**19**) showed activity (Table 1).

3. Biological activity

An experimental model was applied for the vasodepressor effect of the compounds of all three plants separately. The isolated compounds (**1**–**26**) were dissolved in the least amount of ethanol and the solution was diluted to 25% with saline adjusting to 4 ml. Adult Wistar Albino rats weighing 250–300 g were used for the cardiovascular tests. Each animal was anesthetized intraperitoneally (i.p.) with 35 mg/kg sodium pentobarbital (Williams, 1998) and the femoral artery and vein were cannulated separately to monitor the arterial blood pressure and for drug administration. The femoral vein was catheterized for injection of the crude extracts and the isolated diterpenoids separately. Direct blood pressure was recorded from a cannulated femoral artery by connecting to a polygraph (Nihon-Kohden

Table 2
Antibacterial activity (MIC)[a] of compounds **1**, **11**, **12**, **30**, **35**, **36**, and **42**

Compound	Organisms								
	S. aureus	*S. epidermis*	*E. faecalis*	*B. subtilis*	*E. coli*	*P. mirabilis*	*K. pneumonia*	*P. aeruginosa*	*C. albicans*
1	>250	>250	N.A[b]	>250	N.A	N.A	N.A	N.A	N.A
11	6.5	1.5	14	1.5	N.A	N.A	N.A	N.A	N.A
12	10.0	6.0	N.A	3.0	N.A	N.A	N.A	N.A	N.A
30	15.6	15.6	N.A	15.6	N.A	>250	N.A	N.A	N.A
35	N.A	8.0	N.A	N.A	N.A	8.0	N.A	N.A	N.A
36	9.0	18.0	N.A	9.0	N.A	N.A	N.A	N.A	N.A
41	N.A	N.A	N.A	N.A	6.5	N.A	N.A	N.A	N.A

[a] Minimal inhibitory concentrations of the compounds given in μg/ml.
[b] NA = not active (determined by dish-diffusion method).

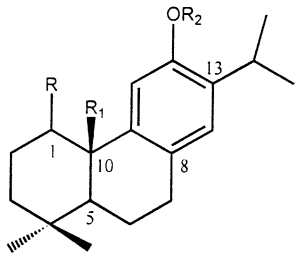
RM 6000, *S. adella*). Heart rates of the animals were calculated from a routine electrocardiogram using pin electrodes as indicated in the literature (Schüssler et al., 1995). The experimental study was carried out with two groups of animals. The control group received ethanol diluted to 25% with saline, while the test group received single compounds intraveneously in doses calculated from a dose response curve. As seen in Table 1, a significant reduction in the direct pressure was observed, together with a slight increase in the heart rate, which did not reach a significant level. The compounds ferruginol (**1**), 3β-hydroxystigmast-5-en-7-one (**7**), 7-oxo-abieta-9,12,14-triene (**14**), stigmast-4-en-3-one (**15**), 4,14-dihydroxysaprorthoquinone (**19**), aethio-pinone (**20**), 4,12 dihydroxysapriparaquinone (**22**) and 6,7-dehydroroyleanone (**25**), and the plant extracts (**I**, **II**, and **III**) respectively, showed activity, while the other samples were not active. Responses of the active compounds and the crude extracts on heart rate, systolic and diastolic (mean) arterial blood pressure were not noted.

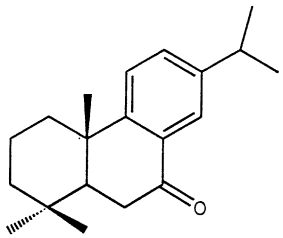
4. Antibacterial compounds

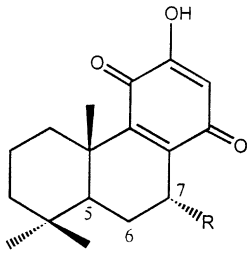
Four other *Salvia* species were investigated as part of the Tubitak–Julich project. From *S. viridis* L. (Ulubelen et al., 2000b) three new diterpenoids, salviviridinol (**27**), viridinol (**28**), and viridone (**29**) together with known compounds 1-oxoferruginol (**30**), ferruginol (**1**), aethiopinone (**20**), and microstegiol (**31**). Two new diterpenoids, 6β-hydroxy-8,15-pimara-dien-1-one (**32**) and 3-oxo-8,11,13-abietatrien-20-oic acid (**33**), were obtained from *S. ceratophylla* L. (Gören et al., 2002) in addition to five known diterpenes salvipisone (**34**), aethiopinone (**20**), candidissiol (**35**) and ferruginol (**1**). *S. caespitosa* Montbret and Aucher ex. Bentham (Ulubelen et al., 2001a) has yielded one new 6β-hydroxyisopimaric acid (**36**) and four known diterpenes isopimaradienal (**37**), ferruginol (**1**), 6β-hydroxyisopimaric acid (**36**), isopimaric acid (**38**), and sandracopimaric acid (**39**). Two new diterpenoids, blephaein (**40**) and *O*-methylpisiferic acid methyl ester (**41**), and eight known compounds, pisiferic acid (**42**), *O*-methylpisiferic acid (**43**), multicaulin (**44**), multiorthoquinone (**45**), demethylmultiorthoquinone (**46**), ferruginol (**1**), horminone (**11**), and 7-acetylhorminone (**12**) were isolated from *Salvia blepharochlaena* Hedge and Hub. Mor. (Ulubelen et al., 2001b). The structures of the new compounds were established by spectral data, including 1D and 2D NMR spectroscopic techniques. The structures of the known compounds were deduced by comparing their spectral data to those of authentic samples.

5. Antibacterial activity

Compounds obtained from the above mentioned four *Salvia* species were dissolved in diluted alcohol (1:10, v/v) and further dilutions made with sterile distilled water. The paper disc diffusion method was used for a preliminary, qualitative evaluation of their antimicrobial effects (NNCLC, 1997). Overnight cultures of bacteria, namely *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538 P, *S. epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 8739, *Proteus mirabilis* ATCC 14153, *Klebsiella pneumonia* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231. Six compounds horminone (**11**), 7-acetylhorminone (**12**), 1-oxo-ferruginol (**30**), 6β-hydroxyisopimaric acid (**36**), candidissiol (**35**) and *O*-methylpisiferic acid methyl ester (**41**) showed activity in one or more strains (Table 2). Ferruginol (**1**) exhibited weak activity as shown in Table 2. The antibacterial activities were comparable with those of commonly used antibiotics against these bacteria. The following values as the levels for susceptibility can be given (NNCLS, 1998): cefoperazone ⩾16 μg/ml, amicain ⩾16μg, kanamycin ⩾16 μg/ml, nitrofurantoin ⩾32 μg/ml, sulfonamides ⩾256 μg/ml.



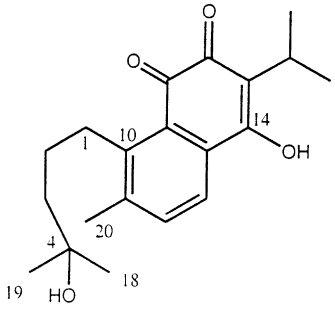


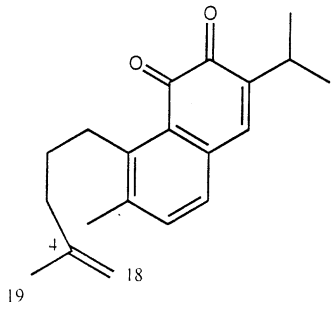


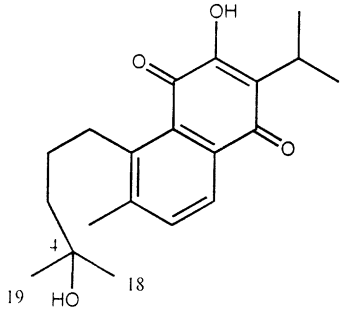
1 R = R_2 = H ; R_1 = Me
30 R = O ; R_1 = Me ; R_2 = H
41 R = H ; R_1 = COOH ; R_2 = Me

14

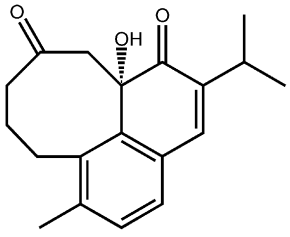
11 R = OH
12 R = OAc
25 R = H ; 6,7 = Δ

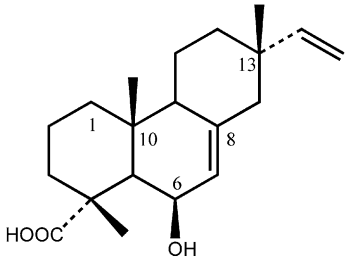




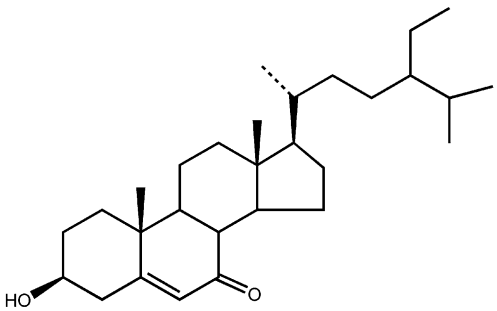


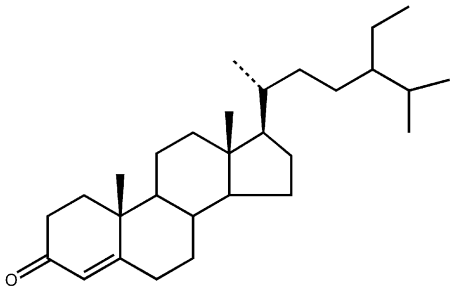
19 **20** **22**





35 **36**





7 **15**

6. Conclusions

Diterpenes obtained from seven *Salvia* species are mostly abietane (**1**, **11**, **12**, **14**, **25**, **30**, **42**), rearranged abietane (**19**, **20**, **22**, **35**), and four isopimarane (**36**, **37**, **38**, **39**) type compounds. Some of them exhibited cardiovascular (**1**, **14**, **19**, **20**, **22**, **25**) and others antibacterial (**11**, **12**, **30**, **35**, **36**, **42**) activities. Two steroidal compounds **7** and **15** also showed cardiovascular activity. This study, in addition to various other studies, justifies the folkloric use of *Salvia* species all around the world. Additional bioactivity tests, especially on cancer cell lines with the compounds obtained from Turkish *Salvia* species are still in progress. The possibility to develop non-toxic drugs from plants is important for the pharmaceutical industry in developing countries, as well as the developed ones. These compounds may serve as useful templates for further structure modification and biological evaluation.

References

Dobrynin, V.N., Kolosov, M.N., Chernov, B.K., Derbentseva, N.A., 1976. Antimicrobial substances of *Salvia officinalis*. Khim. Prir. Soedin 5, 686–687.

Gören, A.C., Topcu, G., Öksüz, S., Kökdil, G., Voelter, W., Ulubelen, A., 2002. Diterpenes from *Salvia ceratophylla*. Nat. Prod. Lett. 16, 47–52.

Hitokato, H., Morozumi, S., Wauke, T., Saiki, S., Kurata, H., 1980. Inhibitory effects of species on growth and toxin production of toxigenic fungi. App. Envir. Microbiol. 39, 818.

Kolak, U.S., Ari, Ş., Birman, H., Hasancebi, S., Ulubelen, A., 2001. Cardioactive diterpenoids from the roots of *Salvia amplexicaulis*. Planta Med. 67, 761–763.

Lin, L.-Z., Wang, X.-M., Huang, Y., Cordell, G.A., 1989. Antibacterial, antituberculous and antiphlogistic activities. Phytochemistry 28, 3542–3543.

Miski, M., Ulubelen, A., Johansson, C., Mabry, T.J., 1983. Antibacterial studies of flavonoids from *Salvia palaestina*. J. Nat. Prod. 46, 874–875.

NCCLS, 1997. Methods for the dilution antimicrobial susceptibility tests for bacteria that grow aerobically, fourth ed. Approved Standard. NCClS document M7-A4, Vol. 17, No. 2. Wayne, PA.

NCCLS, 1998. Performance standards for antimicrobial susceptibility testing; eighth informational supplement, NCCLS document M100-S8, Vol. 18, No. 1, Wayne, PA.

Schüssler, M., Hölzl, J., Fricke, U., 1995. Myocardial effects of flavonoids from *Crataegus* species. Drug Research 45, 842–845.

Topcu, G., Tan, N., Gökdil, G., Ulubelen, A., 1997. Terpenoids from *Salvia glutinosa*. Phytochemistry 45, 1293–1294.

Ulubelen, A., Evren, N., Tuzlaci, E., Johansson, C., 1988. Diterpenoids from the roots of *Salvia hypargeia*. J. Nat. Prod. 51, 1178–1183.

Ulubelen, A., Topcu, G., Tan, N., Lin, L.-J., Cordell, G.A., 1992. Microstegiol, a rearranged diterpene from *Salvia microstegia*. Phytochemistry 31, 2419–2421.

Ulubelen, A., Topcu, G., Eri, C., Sönmez, U., Kartal, M., Kurucu, S., Bozok-Johansson, C., 1994. Terpenoids from *Salvia sclarea*. Phytochemistry 36, 145–147.

Ulubelen, A., Sönmez, U., Topcu, G., Bozok-Johansson, C., 1996. An abietane diterpene and two phenolics from *Salvia forskahlei*. Phytochemistry 42, 145–147.

Ulubelen, A., Topcu, G., Bozok-Johansson, C., 1997. Norditerpenoids and diterpenoids from *Salvia multicaulis* with antituberculous activity. J. Nat. Prod 60, 1275–1280.

Ulubelen, A., Öksüz, S., Kolak, U., Birman, H., Voelter, W., 2000a. Cardioactive terpenoids and a new rearranged diterpene from *Salvia syriaca*. Planta Med. 66, 627–629.

Ulubelen, A., Öksüz, S., Kolak, U., Bozok-Johansson, C., Çelik, C., Voelter, W., 2000b. Antibacterial diterpenes from the roots of *Salvia viridis*. Planta Med. 66, 458–462.

Ulubelen, A., Öksüz, S., Topcu, G., Gören, A.C., Bozok-Johansson, C., Çelik, C., Kökdil, G., Voelter, W., 2001a. A new antibacterial diterpene from the roots of *Salvia caespitose*. Nat. Prod. Lett. 15, 1–8.

Ulubelen, A., Öksüz, S., Topcu, G., Gören, A.C., Voelter, W., 2001b. Antibacterial diterpenes from the roots of *Salvia blepharochlaena*. J. Nat. Prod. 64, 549–551.

Ulubelen, A., Birman, H., Öksüz, S., Topcu, G., Kolak, U., Barla, A., Voelter, W., 2002. Cardioactive diterpenes from the roots of *Salvia eriophora*. Planta Med. 68, 1–4.

Williams, G.H., 1998. Harrison's Principles of International Medicine (Vol. 1), fourteenth ed.

Ayhan Ulubelen is an Emeritus Professor at the Istanbul University Faculty of Pharmacy. She holds a PhD (1958) degree in Analytical Chemistry from Istanbul University. She received a Fulbright scholarship in 1960 to work at the University of Minnesota (1960–1962) and then received an NIH contract to work at the University of Arizona (1963–1967). She became Full Professor at the Chemistry Department of the Faculty of Pharmacy at Istanbul University, and became head of the Department in 1972. She has published over 300 papers on structure determination of natural products, and chapters in Natural Products related books. She served for 4 years in the NATO Grant Commitee as the Chemistry Member, and was a visiting professor at various universities, such as in Tokyo, Würtzburg, Münich, Tubingen, and Texas. She is a member of the Turkish Academy of Sciences (TUBA) and holds a Turkish Scientific and Industrial Research Council (Tubitak) Award. She is an honorary member of the Turkish Chemistry Foundation.