MHz, $CDCl_3$): δ 1.36 (6H, *s*), 1.55 at 2.06 (4H, *m*), 2.38 (3H, *s*), 2.98 (2H, *t*, J = 6 Hz), 3.10 (2H, *t*, J = 5 Hz), 4.36 (2H, *t*, J = 5 Hz), 6.92 (1H, *s*), 7.26 (1H, *d*, J = 8.5 Hz) and 7.70 (1H, *d*, J = 8.5 Hz). ^{13}C NMR (25.2 MHz, $CDCl_3$): δ 18.3 (*q*), 19.5 (*t*), 26.2 (*t*), 26.6 (*t*), 31.4 (*q*), 31.4 (*q*), 34.1 (*s*), 38.8 (*t*), 65.5 (*t*), 108.3 (*d*), 120.2 (*s*) 121.2 (*d*), 123.0 (*s*), 127.3 (*s*), 128.6 (*d*), 128.6 (*s*), 131.3 (*s*), 142.4 (*s*) and 150.5 (*s*). MS *m/z* (rel. int.): 266 $[M]^+$ (71), 251 (100), 236 (14), 221 (16), 210 (9) and 207 (8).

Acknowledgements—The authors are indebted to Dra. Nanuza L. de Menezes for identification of the plants and to the Ministry of Planning (FINEP) and the National Research Council (CNPq) for financial assistance.

REFERENCE

1. Pinto, A. C., Pinchin, R., Zocher, D. H. T. and Lopes, C. C. (1979) *Tetrahedron Letters* 405.

Phytochemistry, Vol. 23, No. 4, pp. 919–921, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

ISOCARNOSOL, A DITERPENE FROM *SALVIA LANIGERA*

HASSAN M. G. AL-HAZIMI, M. S. HASSAN DEEP and GHULAM A. MIANA

Dept. of Chemistry, College of Science, King Saud University, Riyadh, Saudi Arabia

(*Revised received* 30 *August* 1983)

Key Word Index—*Salvia lanigera*; Labiatae; diterpene; isocarnosol.

Abstract—Isocarnosol, a novel diterpene from the leaves of *Salvia lanigera*, has been characterized.

INTRODUCTION

The leaves of *Salvia lanigera* Poir are used as an aromatic tea for a variety of abdominal troubles. A number of diterpenes and diterpene-quinones have been isolated from many *Salvia* species [1–9]. Four diterpene-quinones of the royleanone type have been isolated [10] from *S. lanigera*.

RESULTS AND DISCUSSION

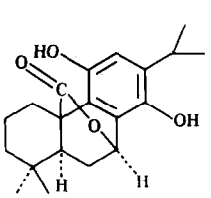
Column chromatography (silica gel) of the petrol extract of the leaves of *S. lanigera* gave a number of fractions. Isocarnosol crystallized out from the chloroform fraction and was recrystallized from methanol to give white crystals, mp 216°. Isocarnosol was given the structure **1** primarily on the basis of ^{1}H NMR data.

Isocarnosol (**1**) $C_{20}H_{26}O_4$ has the same molecular formula as carnosol (**2**) which has been isolated from *Salvia triloba* [11], but the physical properties of the two compounds are different. The IR spectrum of isocarnosol showed a striking resemblance in many of its features to that of carnosol, which exhibits the presence of a 6-membered lactone ring (1730 and 1205 cm^{-1}), an aromatic ring (3050, 1600 and 1500 cm^{-1}) and phenolic hydroxyl groups at 3350 cm^{-1}.

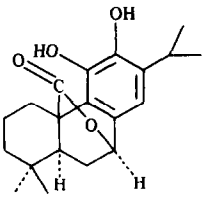
The ^{1}H NMR spectrum of **1** in $CDCl_3$ containing a small amount of DMSO-d_6 indicated the presence of an isopropyl group (δ 1.18, J = 6.7 Hz), two C-methyl (0.85 and 0.89) and a characteristic low field aromatic C–H at 6.63. The aromatic C–H shifted to low field at δ 7.0 in the diacetate derivative indicating its proximity to one of the phenolic hydroxyl groups. The phloroglucinol test for an *o*-diphenol function [12] was negative for isocarnosol, thus leaving the other two isomers (*meta* and *para*) as possibilities. These conclusions were supported by the interpretation of the ^{13}C NMR spectrum of isocarnosol in which the unsubstituted aromatic carbon resonates at δ 111.78. The up-field shift (δ 111.78) for the unsubstituted carbon atom is characteristic of an aromatic carbon adjacent to an oxygen substituent. C-14 in carnosol (**2**) should resonate in the same region as does the same carbon in the diacetate derivative of aethiopinone (**3**) [13] or in carnosolon (**4**) [14] (δ 124.0 and 119.7 respectively).

At this stage the compound was identified as isocarnosol (**1**) or its *meta*-isomer. A structure of the sempervirol type was excluded on biosynthetic grounds as none of the more than 40 diterpene and diterpene-quinones isolated from various *Salvia* species has this structure [15]. However, examination of the IR and NMR data of the oxidation product of **1** showed that this compound was of the *p*-quinonoid type. Thus the IR spectrum of the major product of the bromine oxidation of **1** showed a band at 1660 cm^{-1} (shoulder) typical of the quinonoid structure and an intense band at 1760 cm^{-1} attributable to carbonyl lactone absorption. The appearance of a doublet signal at δ 6.67 ($J \sim$ 1.2 Hz) in the ^{1}H NMR spectrum indicates the structure **5**.

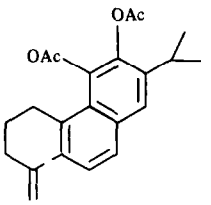
The small coupling constant for proton H-12 ($\sim$ 1.2 Hz) is due to allylic coupling with a hydrogen at C-15, and this situation could not be expected in the *m*-quinonoid type **6** (i.e. H-11 should resonate as a singlet). The ^{13}C NMR spectrum of isocarnosol (Table 1) indicates that one of the C-4 methyl groups is in a 1,3-diaxial relationship with the carboxyl lactone at C-10 and this can happen only if the A/B rings are *trans*-fused [16]. That compounds **1** and **2** have the same absolute configuration



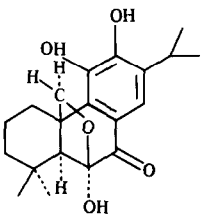
1



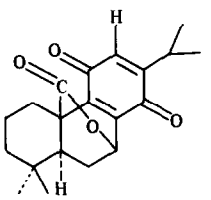
2



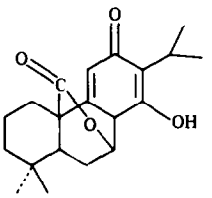
3



4



5



6

was shown by a comparison of the specific rotation of **1** and **2** in accordance with Klyne's rule [16].

Examination of the mass spectrum revealed the presence of fragments at *m/z* 330 $[M]^+$ (13.3%), 286 (100), 271 (53.5), 230 (18.0), 218 (10.6), 216 (20.5) and 204 (23.6). The appearance of a base peak at *m/z* 286 is due to the easy fragmentation of **1** and loss of a CO_2 moiety which is consistent with the dibenzylic position [17] of the lactone ring.

EXPERIMENTAL

The NMR spectra were obtained in $CDCl_3$ on a Jeol 100 MHz with TMS as internal standard. IR and UV spectra were taken in KBr and MeOH respectively. Mps were determined on a hot stage melting point apparatus and were uncorr.

Plant material. The plants were collected in the West Bank area of Jordan river and authenticated by the Department of Botany, King Saud University, Riyadh, Saudi Arabia.

Isolation procedure. Ground leaves (2 kg) were extracted with petrol for 2 weeks. The filtered petrol extract was concd to give a waxy residue (86 g). A portion of the extract was dissolved in petrol and chromatographed on a column of silica gel (200 g). The column was eluted with petrol, petrol–$CHCl_3$ (1:1), $CHCl_3$ and $CHCl_3$–MeOH mixtures. Fractions obtained from $CHCl_3$ afforded crystalline isocarnosol (**1**). It was recrystallized from MeOH to give colourless prisms, mp 216°; $[\alpha]_D^{20} = -80°$ ($CHCl_3$, *c* 0.2%); UV λ_{max}^{MeOH} nm (ε): 235 (22120), 295 (10200), 320 (570); IR ν_{max}^{KBr} cm^{-1}: 3350, 3050, 1730, 1600, 1500, 1450, 1400, 1350, 1205, 1050, 870; ^1H NMR ($CDCl_3$): δ 0.85 (*s*, 3H), 0.89 (*s*, 3H), 1.18 (*d*, *J* = 6.7 Hz, 2 × 3H), 2.48 (*dd*, *J* = 5.0 and 8.0 Hz, 1H), 2.8 (*br*, *d*, 1H), 5.35 (*br d*, H-7), 6.63 (*s*, H-12), 1.4–2.3 (complex signal); ^{13}C NMR: see Table 1; MS *m/z* (rel. int.): see Results and Discussion.

Table 1. ^{13}C NMR spectrum of isocarnosol ($CDCl_3$)

C-1	29.8(*t*)	C-11	*142.61(*s*)
C-2	18.8(*t*)	C-12	111.78(*d*)
C-3	41.0(*t*)	C-13	134.55(*s*)
C-4	28.9(*s*)	C-14	*142.78(*s*)
C-5	45.4(*d*)	C-15	26.8(*s*)
C-6	34.4(*t*)	C-16	22.7(*q*)
C-7	77.4(*d*)	C-17	22.7(*q*)
C-8	131.9(*s*)	C-18	19.7(*q*)
C-9	122.2(*s*)	C-19	31.7(*q*)
C-10	48.4(*s*)	C-20	176.3(*s*)

*Values may be interchangeable.

Oxidation of **1**. Isocarnosol (0.50 g) was dissolved in 40 ml EtOAc by heating. After cooling the soln to room temp, bromine water was added dropwise with stirring. The soln turned green and the excess of bromine was checked by KI/starch paper. The two layers were separated and the EtOAc layer washed with H_2O, $Na_2S_2O_3$ and again with H_2O. The EtOAc layer was dried (Na_2SO_4) and evaporated. TLC examination showed the presence of two spots which were separated to give **5** as the major compound.

Spectral data of **5**. UV λ_{max}^{MeOH} nm (ε): 240 (9300), 275 (8000), 418 (7150); IR ν_{max}^{KBr} cm^{-1}; 3050, 2950, 1760, 1660 (sh), 1250, 800; ^1H NMR ($CDCl_3$): δ 0.87 (*s*, 3H), 0.89 (*s*, 3H), 1.12 (*d*, *J* = 7 Hz, 2 × 3H), 3.09 (*m*, 1H), 5.29 (*m*, H-7), 6.67 (*d*, *J* = 1.2 Hz, H-12).

Acetylation of **1**. Compound **1** (0.5 g) was dissolved in pyridine (5 ml) and 5 ml Ac_2O was added. The soln was left overnight at room temp. after which it was poured onto crushed ice and the precipitated solid filtered. The residue could not be induced to crystallize, but showed a single spot on TLC. IR ν_{max}^{KBr} cm^{-1}: 3050, 2950, 1750, 1390, 1370, 1240, 1170, 1030, 790; ^1H NMR($CDCl_3$): δ 0.84 (*s*, 3H), 0.88 (*s*, 3H), 1.19 (*d*, *J* = 6.8 Hz, 2 × 3H), 3.0 (*septet*, *J* = 6.8 Hz, 1H), 5.4 (*m*, H-7) and 7.0 (*s*, H-12).

Acknowledgements—We are grateful to the King Saud University for financial support through a grant from Research Centre, College of Science. Thanks are also due to Dr. G. Eckhardt, Institute of Organic Chemistry and Biochemistry, University of Bonn, Bonn, Germany for mass spectra.

REFERENCES

1. Gonzalez, A. G., Fraga, B. M., Luis, J. G. and Ravelo, A. G. (1973) *Experientia* **29**, 1471.
2. Escudero, J., Perez, L., Rabanal, R. M. and Valverde, S. (1982) *Phytochemistry* **22**, 585.
3. Brieskorn, C. H., Fuchs, A., Bredenberg, G. B., McChensey, J. D. and Wenkert, E. (1964) *J. Org. Chem.* **29**, 2293.
4. Vlasova, C. F., Romanova, A. S., Perelson, M. F. and Ban'lovskii, A.I. (1969) *Khim. Prir. Soedin* 317.
5. Watson, W. H. and Taira, Z. (1976)*Tetrahedron Letters* 2501.
6. Baojin, Y., Mingkun, Q., Guowei, Q. and Zhengxiong, C. (1981) *Yaoxue Xuebao* **16**, 837. *Chem. Abst.* (1982) **96**,

177900b.
7. Kakisawa, H., Hayashi, T. and Yamazaki, T. (1969) *Tetrahedron Letters* 301.
8. Hayashi, T., Handa, T., Ohashi, M. and Kakisawa, H. (1971) *Chem. Commun.* 541.
9. Ulubelen, A. and Miski, M. (1980) *J. Nat. Prod.* **44**, 119.
10. Saleh, M. R. I., Sabri, N. and El-Masry, S. (1978) *Egypt J. Pharm. Sci.* **19**, 313.
11. Ulubelen, A., Ozturk, S. and Isildatici, S. (1968) *J. Pharm. Sci.* 1037.
12. Schroder, H. A. (1967) *J. Chromatogr.* **30**, 537.
13. Boya, M. T. and Valverde, S. (1981) *Phytochemistry* **20**, 1367.
14. Yoshizaki, V. F., Ruedi, P. and Eugster, C. H. (1979) *Helv. Chim. Acta* **62**, 2754.
15. Al-Hazimi, H. M. G. and Miana, G. A. (1984) *J. Nat. Prod.* (submitted for publication).
16. Narayanan, C. R. and Linde, H. (1965) *Tetrahedron Letters* 3647.
17. Hodges, R., Cambie., R. C. and Joblin, K. N. (1970) *Org. Mass Spectrom.* **3**, 1473.

Phytochemistry, Vol. 23, No. 4, pp. 921–923, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

24α-METHYL-5α-CHOLEST-7-EN-3β-OL FROM SEED OIL OF *HELIANTHUS ANNUUS*

TARO MATSUMOTO, MASAYUKI NAKAGAWA and TOSHIHIRO ITOH

College of Science and Technology, Nihon University, 1-8, Kanda Surugadai, Chiyoda-ku, Tokyo, 101 Japan

(*Received* 6 *September* 1983)

Key Word Index—*Helianthus annuus*; Compositae; seeds; 24-alkylsterols; 24α-methyl-5α-cholest-7-en-3β-ol.

Abstract—24-Methyl-5α-cholest-7-en-3β-ol (24-methyllathosterol) isolated from the seed oil of *Helianthus annuus* was shown to have 24α-configuration by ^{1}H NMR spectroscopy. The stereochemistry at C-24 of some other 24-alkylsterols isolated from this plant material also was determined.

24-Methylcholest-5-en-3β-ol (24-methylcholesterol, **6**) [1–3] and 24-methyl-*trans*-22-dehydrocholesterol (**9**) [4–6] found in higher plants often consist of a mixture of epimers at C-24, i.e. campesterol (24α) and 22-dihydrobrassicasterol (24β) for **6**, and *trans*-22-dehydrocampesterol (24α) and brassicasterol (24β) for **9**. 24-Methyl-5α-cholest-7-en-3β-ol (24-methyllathosterol, **1**), a Δ^7-analog of **6**, also occurs in some higher plants including Compositae plants such as *Helianthus annuus* and *Carthamus tinctorius* [7]. However, the stereochemistry at C-24 of this sterol (**1**) from these higher plants remains undetermined to the best of our knowledge. Therefore, we have conducted here the determination of C-24 stereochemistry with the aid of 400 MHz ^{1}H NMR spectroscopy for sterol **1** and some other 24-alkylsterols isolated from the seed oil of *H. annuus* and have undertaken thorough analysis of the total sterol fraction of this plant material.

The Δ^5- (R_f = 0.33, fraction A, 291 mg) and Δ^7- (R_f = 0.31, fraction B, 50 mg) sterol fractions, separated by silica gel TLC from the unsaponifiable lipid (1.5 g) of *H. annuus* seed oil (160 g), were acetylated, and the resulting acetate fractions were subjected to silver nitrate–silica gel TLC. The acetylated fraction A gave three further fractions. Fraction A-1 from the least polar band (R_f = 0.60) was a mixture of the acetates of **6** and 24-ethylcholesterol (**7**). Fraction A-2 from the band of R_f = 0.44 was 24-ethyl-*trans*-22-dehydrocholesteryl (**8**) acetate. Fraction A-3 from several bands of R_f = 0.1–0.4 consisted of the acetates of 24-methyl-*trans*-22-dehydrocholesterol (**9**), 24*Z*-ethylidenecholesterol (isofucosterol, **11**) and 24-methylene-25-methylcholesterol (**12**). The acetylated fraction B separated into two fractions on argentation TLC. Fraction B-1 from the least polar band (R_f = 0.60) consisted of the acetates of **1** and 24-ethyllathosterol (**2**). Fraction B-2 from several bands of R_f = 0.1–0.4 was a mixture of the acetates of 24-methylenelathosterol (episterol, **3**), 24*Z*-ethylidenelathosterol (Δ^7-avenasterol, **4**) and 24-ethyl-24(25)-dehydrolathosterol (peposterol, **5**). The identifications were performed by GC and GC/MS analyses of the acetate derivatives. Acetate fraction A-2 was then hydrolysed to give free sterol **8** (mp 163–166°). Acetate fractions A-1 and B-1 were also hydrolysed and further fractionated by reverse-phase HPLC. Fraction A-1, on HPLC, yielded **6** (RR_t = 1.10, mp 145–148°) and **7** (RR_t = 1.18, mp 136–140°), and fraction B-1 gave **1** (RR_t = 1.09, mp 144–146°) and **2** (RR_t = 1.18, mp 144–148°).

In order to determine the configuration at C-24 of **1** as well as **2** and **6–8**, these sterols were subjected to 400 MHz ^{1}H NMR spectroscopy. Table 1 shows the chemical shifts of the methyl group signals of these sterols accompanied with three authentic sterols, 24β-methyllathosterol, 24α-ethyl-*trans*-22-dehydrocholesterol (stigmasterol) and its 24β-epimer (poriferasterol), for which assignments were