Phytochemistry, Vol 25, No 1, pp. 272 274, 1986.
Printed in Great Britain

0031-9422/86 $3.00 + 0.00

EPOXYSALVIACOCCIN, A NEO-CLERODANE DITERPENOID FROM *SALVIA PLEBEIA*

MARÍA C. GARCÍA-ALVAREZ, MASHOODA HASAN, ANTONIO MICHAVILA*, FRANCISCO FERNÁNDEZ-GADEA* and BENJAMÍN RODRÍGUEZ*

Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan; *Instituto de Química Orgánica, CSIC., Juan de la Cierva 3, 28006-Madrid, Spain

(*Received* 29 *March* 1985)

Key Word Index—*Salvia plebeia*; Labiatae; diterpenoids; neo-clerodane derivatives; epoxysalviacoccin; salviacoccin.

Abstract—A new neo-clerodane derivative, epoxysalviacoccin, has been isolated from the aerial part of *Salvia plebeia*, together with the previously known diterpenoid salviacoccin. The structure of epoxysalviacoccin, 2β,3β; 15,16-diepoxy-10β-hydroxy-neo-cleroda-7,13(16),14-triene-17,12*R*; 18,19-diolide, has been established by spectroscopic means and by partial synthesis from salviacoccin.

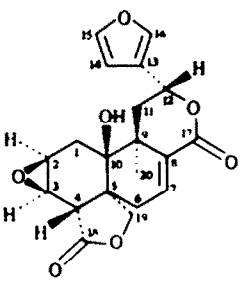
INTRODUCTION

In our search for new natural substances in the *Salvia* genus [1–3], we have examined the aerial part of *S. plebeia* R. Br., a species in which some flavonoids [4, 5] and two phenolic derivatives [6, 7] have been previously found. From this plant we have now isolated a novel diterpenoid, epoxysalviacoccin, the structure of which has been shown to be 2β,3β; 15,16-diepoxy-10β-hydroxy-neo-cleroda-7,13(16),14-triene-17,12*R*; 18,19-diolide (**1**) by chemical and spectroscopic means. In addition, the previously known diterpenoid salviacoccin (**2**) [8] has also been isolated from the same source.

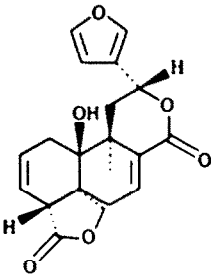
RESULTS AND DISCUSSION

Epoxysalviacoccin (**1**), $C_{20}H_{20}O_7$, showed absorption in its IR spectrum consistent with the presence of a furan ring (3160, 3110, 1505, 877 cm^{-1}), a γ-lactone group (1770 cm^{-1}), another lactone group, probably an α,β-unsaturated δ-lactone (1720, 1670 cm^{-1}), and a hydroxyl group (strong and sharp band at 3510 cm^{-1}). Its ^1H NMR spectrum (Table 1) was almost identical with that reported for salviacoccin (**2**) [8]. In fact, the only difference between the spectra of compounds **1** and **2** was the presence in the former of two signals due to the protons of an α,β-disubstituted oxirane ring (δ3.58, *d* (*br*), J = 4.0 Hz, and 3.63, *dt*, J_1 = 4.0 Hz, J_2 = 1.8 Hz) instead of the C-2 and C-3 olefinic protons of salviacoccin (**2**) [8]. NOE experiments based on irradiating the signal of the C-20 methyl group of epoxysalviacoccin (**1**, δ0.97), showed that the following signals were affected: δ1.75, *dd* (axial C-11α proton, 8% enhancement), 2.34, *dd* (axial C-1α proton, 5% enhancement), 4.17, *d* (H-19A, 9% enhancement) and 4.31, *dd* (H-19B, 5% enhancement). No NOE was observed in the signal of the C-12 proton. These results established a *cis* relationship between the C-20 methyl group, the C-19 methylene grouping and the C-1 and C-11 axial protons, and also showed that the tertiary hydroxyl group must be at the C-10β position and that the oxirane ring must be attached to the C-2 and C-3 carbon atoms, since the axial C-1α proton appeared as a double doublet (J_{gem} = 15.3 Hz, J_{vic} = 1.8 Hz). In addition, the NOE experiment established a 12β configuration for the hydrogen atom at the terminus of the δ-lactone function. The δ-lactone must be in an $^{8,12}B$ conformation, in which there is an H-11α–H-12β *trans* diaxial arrangement to account for the observed NOE between the C-20 methyl group and the C-11α proton and also the $J_{11\alpha,12\beta}$ value of 11.9 Hz. An alternative structure with an H-12α configuration in an $^8C_{12}$ conformation of the δ-lactone ring is not compatible with the above results, since in this case the axial C-11β proton and the C-20α methyl group are *trans* and, consequently, no NOE should be observed between these protons, but an NOE enhancement should be expected on the signal of the axial C-12α proton [1, 9 and refs therein].

The oxirane ring of epoxysalviacoccin (**1**) must be β-oriented, since the coupling values between the C-2 and C-1 protons ($J_{2,1\alpha} = J_{2,1\beta}$ = 1.8 Hz) were only compatible with a 2β,3β-oxirane configuration [10] (see the molecular model of epoxysalviacoccin). Moreover, the C-4β proton of the new diterpenoid (**1**) showed a broad singlet signal (δ3.33, $W_{1/2}$ = 1.82 Hz), with a vicinal coupling value ($J_{4\beta,3\alpha}$) less than 0.3 Hz. Irradiation at δ3.58 (H-3α)



1



2

Table 1. ^{1}H NMR data of compound 1 (300 MHz, pyridine-d_5, TMS as int. standard, J values in Hz)*

H		H	
1α	2.34 *dd*	11β	2.91 *dd*
	$J_{1\alpha,1\beta}$ = 15.3, $J_{1\alpha,2\alpha}$ = 1.8		$J_{11\beta,12\beta}$ = 2.7
1β	2.27 *dd*	12β	5.27 *dd*
	$J_{1\beta,2\alpha}$ = 1.8		
2α	3.63 *dt*	14	6.71 *dd*
	$J_{2\alpha,3\alpha}$ = 4.0		$J_{14,15}$ = 1.7, $J_{14,16}$ = 0.4
3α	3.58 *d* (*br*)	15	7.67 *t*
	$J_{3\alpha,4\beta}$ < 0.3		$J_{15,16}$ = 1.7
4β	3.33 *s* (*br*)	16	7.83 *dd*
6α	2.39 *dd*	19A†	4.17 *d*
	$J_{6\alpha,6\beta}$ = 18.6, $J_{6\alpha,7}$ = 4.9		$J_{19A,19B}$ = 8.9
6β	2.51 *dt*	19B‡	4.31 *dd*
	$J_{6\beta,7}$ = $J_{6\beta,19B}$ = 2.1		$J_{19B,6\beta}$ = 2.1
7	6.60 *dd*	Me-20	0.97 *s*
11α	1.75 *dd*	OH§	4.60 *s*
	$J_{11\alpha,11\beta}$ = 13.9, $J_{11\alpha,12\beta}$ = 11.9		

*All these assignments were confirmed by double resonance experiments.
†*Endo* hydrogen with respect to ring B.
‡*Exo* hydrogen with respect to ring B.
§Disappeared after addition of D_2O.

caused a noticeable sharpening (now $W_{1/2}$ = 1.51 Hz) of the signal of the C-4β proton (δ3.33), in complete agreement with an H–C-4–C-3–H dihedral angle close to 90°.

Final proof that epoxysalviacoccin has the structure and absolute configuration of neo-clerodane [11] dipicted in formula 1 was obtained by reaction of salviacoccin (2) with *m*-chloroperbenzoic acid (see Experimental), which yielded a compound identical in all respects (mp, mmp, $[\alpha]_D$, TLC, IR, ^{1}H NMR and MS) with natural epoxysalviacoccin (1).

EXPERIMENTAL

Mps: uncorr; general details: see refs [1–3, 8]. Plant materials were collected in September 1984 at the University Campus, Islamabad, Pakistan.

Extraction and isolation of the diterpenoids. Dried and finely powdered *S. plebeia* aerial parts (1.2 kg) were extracted with Me_2CO (15 l.) at room temp. for 1 week. After filtration, the solvent was evaporated and the residue (27 g) subjected to dry CC over silica gel (450 g, Merck No. 7734, deactivated with 10% H_2O). The column was eluted with *n*-hexane and *n*-hexane–EtOAc mixtures. The fractions eluted with *n*-hexane–EtOAc (4:1) yielded a white residue (4 g) of a mixture of ursolic acid and diterpenoids 1 and 2. This mixture was treated with ethereal CH_2N_2 in Et_2O–MeOH (1:2) soln for 3 hr at 10°. After evaporation of the solvents, the residue was crystallized from EtOAc, yielding 1.3 g of a mixture of 1 and 2. CC (silica gel, $CHCl_3$–MeOH, 32:1) gave, in order of elution, epoxysalviacoccin (1, 120 mg) and salviacoccin (2, 1 g). The previously known compound 2 was identified by its physical (mp, $[\alpha]_D$) and spectroscopic (IR, ^{1}H NMR, MS) data and by comparison (mmp, TLC) with an authentic sample [8].

Epoxysalviacoccin (1). Mp 197–199° (MeOH); $[\alpha]_D^{18}$ −40.2° (pyridine; *c* 0.241); IR ν_{max}^{KBr} cm^{-1}: 3510 (hydroxyl group), 3160, 3110, 1505, 877 (furan ring), 1795, 1770 (γ-lactone), 1720, 1670 (*exo* α,β-unsaturated δ-lactone), 3000, 2935, 1430, 1365, 1270, 1250, 1160, 1140, 1055, 1040, 1015, 1010, 1003, 822, 790, 740, 650; UV λ_{max}^{MeOH} nm (log ε): 217 (3.87), 225 (3.63), 230 (3.40), 235 (3.20), 240 (3.00), 250 (2.70); ^{1}H NMR (300 MHz, pyridine-d_5): see Table 1; EIMS (direct inlet) 75 eV, *m/z* (rel. int.): 372 [M]$^+$ (44), 354 (17), 326 (56), 245 (90), 182 (19), 155 (34), 131 (29), 129 (29), 128 (27), 115 (25), 95 (100), 94 (51), 91 (63), 81 (71), 65 (46), 53 (51), 43 (63), 41 (70). (Found: C, 64.43; H, 5.46. $C_{20}H_{20}O_7$ requires: C, 64.51; H, 5.41%.)

Epoxysalviacoccin (1) *from salviacoccin* (2). Salviacoccin (2, 30 mg, 0.080 mmol) was treated with *m*-chloroperbenzoic acid (15 mg, 0.087 mmol) in CH_2Cl_2 soln for 24 hr at 4°. Work-up in the usual manner yielded 26 mg of a compound identical (mp, mmp, $[\alpha]_D$, TLC, IR, ^{1}H NMR and MS) in all respects with natural epoxysalviacoccin (1).

Acknowledgements—We thank Dr. Nazir Chaudhary, Department of Biology, Quaid-i-Azam University, Islamabad, Pakistan, for the botanical classification of the plant material, the Embassy of Spain in Pakistan for mail facilities, and Professor Sheikh Imtiaz Ali, Vicechancellor of the Quaid-i-Azam University, for his support. This work was supported in part by the Spanish 'Comisión Asesora de Investigación Científica y Técnica'.

REFERENCES

1. Rodríguez, B., Pascual, C. and Savona, G. (1984) *Phytochemistry* **23**, 1193.
2. Rodríguez, B., Fernández-Gadea, F. and Savona, G. (1984) *Phytochemistry* **23**, 1805.
3. Michavila, A., Fernández-Gadea, F. and Rodríguez, B. (1986) *Phytochemistry* **25**, 266.
4. Gupta, H. C., Ayengar, K. N. N. and Rangaswami, S. (1975)

Indian J. Chem. 13, 215.
5. Yang, T. H. and Chen, K. T. (1972) *J. Chin. Chem. Soc. (Taipei)* 19, 131.
6. Powell, R. G. and Plattner, R. D. (1976) *Phytochemistry* 15, 1963.
7. Plattner, R. D. and Powell, R. G. (1978) *Phytochemistry* 17, 149.
8. Savona, G., Bruno, M., Paternostro, M., Marco, J. L. and Rodríguez, B. (1982) *Phytochemistry* 21, 2563.
9. Fayos, J., Farnández-Gadea, F., Pascual, C., Perales, A., Piozzi, F., Rico, M., Rodríguez, B. and Savona, G. (1984) *J. Org. Chem.* 49, 1789.
10. Rodríguez, M.-C., Barluenga, J., Savona, G., Piozzi, F., Servettaz, O. and Rodríguez, B. (1984) *Phytochemistry* 23, 1465.
11. Rogers, D., Unal, G. G., Williams, D. J., Ley, S. V., Sim, G. A., Joshi, B. S. and Ravindranath, K. R. (1979) *J. Chem. Soc. Chem. Commun.* 97.

Phytochemistry, Vol. 25, No. 1, pp 274–276, 1986
Printed in Great Britain

0031-9422/86 $3.00 + 0.00

TRITERPENOIDS OF *CNIDOSCULUS URENS*

J. BHATTACHARYYA and CYMONE B. BARROS

Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, 58.000 João Pessoa, Paraíba, Brazil

(*Received* 15 *April* 1985)

Key Word Index—*Cnidosculus urens*; Euphorbiaceae; β-amyrin cinnamate; germanicol acetate; lupeol acetate; β-amyrin; lupeol; germanicol.

Abstract—The ethanol extract of *Cnidosculus urens* yielded β-amyrin cinnamate and germanicol acetate. Also, using ^{13}C NMR spectroscopy, mixtures of β-amyrin, germanicol and lupeol as well as their acetates were identified.

Preliminary pharmacological studies on the ethanol extract of *Cnidosculus urens* (Euphorbiaceae), a small shrub used in popular medicine [1] showed acetylcholine-like smooth muscle stimulatory effect [2]. The chloroform-soluble part of the ethanol extract, upon further fractionation yielded β-amyrin cinnamate and germanicol acetate along with two apparently homogeneous materials, A and B, which were identified as intimate mixtures of β-amyrin, germanicol and lupeol and their corresponding acetates, respectively, by ^{13}C NMR spectral analysis. This report demonstrates yet another example of the usefulness of ^{13}C NMR spectroscopy in routine identification of relatively common plant products in mixtures which would, otherwise, be a very laborious and wasteful exercise.

The ^{13}C NMR spectrum of β-amyrin cinnamate (1) showed 35 signals for 39 carbons in the molecule. The signals at δ16.9 (two *q*), 23.7 (one *t*, one *q*), 128.0 (two *d*) and 128.8 (two *d*) represent two carbons each. The spectrum showed 30 signals identical to those of the carbons of β-amyrin acetate [3]. However, in place of the acetyl signals, the spectrum showed a singlet at δ166.7 (CO), two doublets at 118.8 (α-CH=) and 144.2 (β-CH=), a singlet a 134.7 (C-1′) and two doublets at 128.0 (C-2′ and C-6′ or C-3′ and C-5′) and 128.2 (C-3′ and C-5′ or C-2′ and C-6′) and a doublet at 130 (C-4′). These signals can be accounted for by the presence of a *trans*-cinnamate moiety instead of an acetate at C-3. The presence of the *trans*-cinnamate moiety is also confirmed by the appearance of two 1H doublets at δ7.66 (J = 16 Hz) and 6.46 (J = 16 Hz) in addition to a 5H multiplet at 7.25–7.55 in the ^{1}H NMR spectrum. Moreover, the alkaline hydrolysis of the compound furnished β-amyrin (3) and cinnamic acid, as expected [4]. Germanicol acetate (4) was identified by comparison of the ^{13}C NMR and other spectral data with those published in the literature [5–7].

The material A, after several crystallizations gave a single spot on a TLC plate but did not show a sharp mp. The molecular weight ($[M]^+$ at m/z 426) of the material corresponded to the formula $C_{30}H_{50}O$. The material A was characterized with the help of ^{13}C NMR spectroscopy. The signals for the olefinic carbons in the ^{13}C NMR spectrum of the pentacyclic triterpenoids are very characteristic and helpful in identifying this type of compound. For example, C-12 and C-13 of the Δ^{12}-oleananes appear at δ121.7 (*d*) and 145.0 (*s*), respectively; the C-18 and C-19 of Δ^{18}-oleananes appear at 142.7 (*s*) and 129.8 (*d*), respectively, and the C-20 and C-29 of $\Delta^{20,29}$-lupanes appear at 150.6 (*s*) and 109.2 (*t*), respectively, which permits the identification of these types of compounds in a mixture with the help of the complete and partial proton noise decoupled ^{13}C NMR spectra. Thus, the olefinic region of the ^{13}C NMR spectrum of the material A showed signals at 150.6 (*s*), 145.2 (*s*), 142.8 (*s*), 129.8 (*d*), 121.8 (*d*) and 109.4 (*t*) which gave an indication