EXPERIMENTAL

Enzymatic extraction of pectic polysaccharides. Sliced tissues (0.5 mm thick) of onion bulb were repeatedly washed with 50 mM ammonium acetate buffer (pH 5.5). After excess buffer soln was removed with filter paper, 2.5 g of the tissues were placed in 100 ml Erlenmeyer flasks containing 25 ml of 50 mM ammonium acetate buffer, pH 5.5 (for PL) or pH 4.5 (for PG) and 20 units of PL or 500 units of PG ex. *Aspergillus japonicus* [7]. For exhaustive extraction 5 g of tissue in 100 ml of 50 mM ammonium acetate buffer, pH 5.5 were treated with 50 units of PL at 30°. After 7 hr the reaction mixtures were filtered and the residue repeatedly washed with buffer. The tissues were then suspended in 100 ml of buffer and incubated with an additional 25 units of PL for another 7 hr. The solubilized material was removed from the tissue by filtration and washing. The third extraction was carried out in the same manner with a further 25 units of PL.

Analysis of pectic polysaccharides. The galacturonide content was estimated by the *m*-hydroxydiphenyl method of ref. [8]. Total uronides were determined by the method of ref. [9]. The neutral sugar content was estimated by the anthrone method [10] using galactose as standard.

Glycosyl linkage compositions were determined by GC/MS analysis of $NaBD_4$-reduced pectic polysaccharides as described previously [4].

Acknowledgements—Grateful acknowledgement is made to M. Sasaki and N. Nunomura for assistance with the GC/MS data and Youko Mogi for technical assistance.

REFERENCES

1. Rees, D. A. and Wight, A. W. (1971) *J. Chem. Soc. B* 1366.
2. Rees, D. A. and Wight, A. W. (1969) *Biochem. J.* **115**, 431.
3. Darvill, A. G., McNeil, M. and Albersheim, P. (1978) *Plant Physiol.* **62**, 418.
4. Ishii, S. (1981) *Phytochemistry* **20**, 2329.
5. Sen, S. K., Chatterjee, B. P. and Rao, C. V. N. (1971) *J. Chem. Soc. C* 1788.
6. Mankarios, A. T., Hall, M. A., Jarvis, M. C., Threlfall, D. R. and Friend, J. (1980) *Phytochemistry* **19**, 1731.
7. Ishii, S. (1976) *Phytopathology* **66**, 281.
8. Blumenkrantz, N. and Asboe-Hansen, G. (1973) *Analyt. Biochem.* **54**, 484.
9. McCready, R. M. and McComb, E. A. (1952) *Analyt. Chem.* **24**, 1986.
10. Dische, Z. (1962) *Methods in Carbohydrate Chemistry* (Whistler, R. L. and Wolfrom, M. L., eds.) Vol. 1, p. 477. Academic Press, New York.

Phytochemistry, Vol. 21, No. 3, pp. 780–782, 1982.
Printed in Great Britain.

0031-9422/82/030780–03$03.00/0

INOSITOL ANGELATES FROM *INULA CAPPA*

FERDINAND BOHLMANN, MANIRUDDIN AHMED and JASMIN JAKUPOVIC

Institute for Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, West Germany

(*Received* 10 *July* 1981)

Key Word Index—*Inula cuppa*; Compositae; inositol and myoinositol esters.

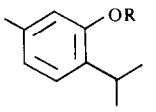
Abstract—The aerial parts of *Inula cappa* afforded four inositol tetra angelates.

From the aerial parts of *Inula cappa* DC. some unusual flavones have been isolated [1]. A re-investigation afforded in addition to the thymol and isothymol derivatives **1–4**, β-farnesene, squalene and caryophyllenepoxide, four angelates, which could only be separated with difficulty. Careful ^{1}H NMR investigation of these esters (**6** and **7** could not be separated completely) led to the structures **5–8** (Table 1). The presence of tetra angelates clearly followed from the ^{1}H NMR signals, as did the presence of two free hydroxyls. As the molecular formula of each compound was the same, **5–8** were isomeric compounds. Spin decoupling allowed the assignment of the sequences of the neighbouring oxygen functions, as the signals of those protons under the ester function were clearly shifted downfield. The stereochemistry was deduced from the observed couplings. Obviously **5** and **6** were derivatives of inositol, while **7** and **8a** were isomeric tetra angelates of myoinositol. Saponification of **5** afforded L-inositol, which seems to be widespread in the Compositae [2], while esters have not been reported. *I. cappa* belongs to the group of species, which lacks sesquiterpene lactones. These seem to be replaced by thymol derivatives [3, 4].

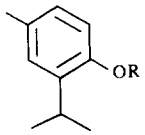
Table 1. ^{1}H NMR spectral data of compounds **5–7** and **8a** and **8b** (400 MHz, $CDCl_3$, TMS as int. standard)

	5	6	7	8a	8b
H-1	5.62 *dd*	4.28 *dd*(*br*)	5.12 *dd*	3.98 *ddd*	5.32 *dd*
H-2	5.60 *dd*	5.34 *dd*	4.14 *dd*(*br*)	5.31 *dd*	5.67 *dd*
H-3	5.52 *dd*	5.66 *dd*	5.28 *dd*	3.84 *ddd*	5.37 *dd*
H-4	4.24 *dd*	4.10 *dd*(*br*)	5.43 *dd*	5.56 *dd*	5.75 *dd*
H-5	5.24 *dd*	5.54 *dd*	4.06 *dd*(*br*)	5.19 *dd*	5.25 *dd*
H-6	4.27 *dd*	5.60 *dd*	5.78 *dd*	5.78 *dd*	5.81 *dd*
OAng	6.18 *qq*	6.20–6.03 *m*		6.19 *qq*	6.18 *m*
	6.16 *qq*			6.17 *qq*	6.07 *m* (3H)
H-3′	6.07 *m*(2H)			6.09 *qq*(2H)	
H-4′	2.03 *dq*	2.05–1.95 *m*		2.04 *dq*	2.03 *d*(*br*)
	2.01 *dq*			2.01 *dq*	1.92 *dq*
	1.94 *dq*			1.93 *dq*	1.90 *dq*
	1.91 *dq*			1.91 *dq*	1.89 *dq*
H-5′	1.98 *dq*	1.95 *s*(*br*), 1.86 *s*(*br*)(6H), 1.84 *s*(*br*)		2.02 *s*(*br*)	2.03 *s*(*br*)
	1.93 *dq*			1.93 *s*(*br*)	1.80 *dq*
	1.85 *dq*			1.83 *dq*	1.78 *dq*
	1.77 *dq*			1.76 *dq*	1.74 *dq*
OH	2.35 *m*	2.74 *d*(*br*), 2.62 *d*(*br*),	2.58 *d*(*br*)	2.58 *d*(*br*)	—
			2.52 *d*(*br*)	2.53 *d*(*br*)	
OAc	—	—	—	—	1.97 *s*, 1.96 *s*

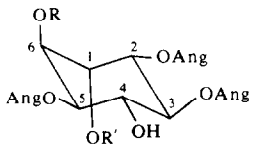
J (Hz): Compound **5**: 1, 2 = 1, 6 = 3.5; 2, 3 = 3, 4 = 4, 5 = 10; 5, 6 = 3.5; compound **6**: 1, 2 = 3;1, 6 = 3.5; 2, 3 = 10.5; 3, 4 = 4, 5 = 10; 5, 6 = 10; 1, OH ~ 2; 4, OH ~ 7; compound **7**: 1, 2 = 3, 4 = 4, 5 = 10; 1, 6 = 5, 6 = 3; 2, OH = 5, OH ~ 5; compound **8a**/**8b**: 1, 2 = 2, 3 = 3, 4 = 4, 5 = 10; 1, 6 = 5, 6 = 3 (**8a**: 1, OH = 6.5; 3, OH = 5); OAng: 3′, 4′ = 7; 3′, 5′ = 4′, 5′ = 1.5.



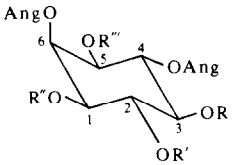
1 R = H
2 R = *i*-Bu



3 R = H
4 R = *i*-Bu



	5	6
R	H	Ang
R′	Ang	H



	7	8a	8b
R	Ang	H	Ac
R′	H	Ang	Ang
R″	Ang	H	Ac
R‴	H	Ang	Ang

EXPERIMENTAL

The air dried aerial parts (150 g), collected near Delhi (voucher deposited at the Botanical Survey of India), were extracted with Et_2O–petrol (1:2) and the resulting extract was separated by CC (Si gel) followed by repeated TLC (Si gel). Known compounds were identified by comparing the ^{1}H NMR spectra with those of authentic material. The less polar part afforded 10 mg β-farnesene, 10 mg squalene, 10 mg 1β, 10α-epoxy-1,10-dihydrocaryophyllene, 3 mg **1**, 4 mg **2**, 3 mg **3** and 3 mg **4**, while the more polar fractions gave a mixture of **5–8**. Repeated TLC (C_6H_6–CH_2Cl_2–Et_2O, 1:1:1 and C_6H_6–CH_2Cl_2–Et_2O, 2:2:1) afforded 12 mg **5**, 15 mg **6** and **7** (*ca* 1:1) and 8 mg **8a**.

L-Inositol-1, 2, 3, 5-*tetra angelate* (**5**). Colourless gum,

IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3610, 3420 (OH), 1725, 1650 (C=CCO$_2$R); MS *m/z* (rel. int.): 508 [M]$^+$ (0.3), 490 [M − H$_2$O]$^+$ (4), 408.178 [M − RCO$_2$H]$^+$ (6) ($C_{21}H_{28}O_8$), 83 [C_4H_7CO]$^+$ (100), 55 [83 − CO]$^+$ (41);

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\ nm}{+4.4 \quad +5.0 \quad +5.3 \quad +8.4}\ (CHCl_3;\ c = 1.0).$$

Saponification (MeOH–H$_2$O–KOH, 70°) afforded L-inositol.

L-Inositol-2, 3, 5, 6-tetra angelate and myoinositol-1, 3, 4, 6-tetra angelate. (**6** *and* **7**). Colourless gum, which could not be separated into its constituents, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3610, 3480 (OH), 1720, 1650 (C=CCO$_2$R); MS *m/z* (rel. int.): 508 [M]$^+$ (0.3), 490 [M–H$_2$O]$^+$ (2), 408.178 [M–RCO$_2$H]$^+$ (5) ($C_{14}H_{28}O_8$) 83 [C_4H_7CO]$^+$ (100), 55 [83 − CO]$^+$ (44).

Myoinositol-2, 4, 5, 6-tetra angelate (**8a**). Colourless gum, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3610, 3440 (OH), 1730, 1250 (C=CCO$_2$R); MS *m/z* (rel. int.): 508 [M]$^+$ (0.3), 490 (4), 408.178 [M − RCO$_2$H]$^+$ (6) ($C_{21}H_{28}O_8$), 83 [C_4H_7CO]$^+$ (100), 55 [83 − CO]$^+$ (40);

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\ nm}{+3.4 \quad +3.8 \quad +4.0 \quad +6.0}\ (CHCl_3;\ c\ 0.74).$$

8 mg **8a** were heated with 0.1 ml Ac_2O for 1 hr at 70°. TLC (CH_2Cl_2–C_6H_6–Et_2O, 2:2:1) afforded 4 mg **8b**, colourless gum, MS *m/z* (rel. int.): 592 [M]$^+$ (0.2), 532 [M − HOAc]$^+$ (4), 492 [M − RCO$_2$H]$^+$ (10), 83 [C_4H_7CO]$^+$ (100).

Acknowledgements—We thank Dr. R. Gupta, University of Delhi, for plant material and the Deutsche Forschungsgemeinschaft for financial support.

REFERENCES

1. Baruah, N. C., Sharma, R. P., Thyagarajan, G. and Herz, W. (1979) *Phytochemistry* **18**, 2003.
2. Plouvier, V. (1963) in *Chemical Plant Taxonomy* (Swain, T., ed.) p. 323. Academic Press, London.
3. Bohlmann, F. and Zdero, C. (1977) *Phytochemistry* **16**, 1243
4. Bohlmann, F., Mahanta, P. K., Jakupovic, J., Rastogi, R. C. and Natu, A. A. (1978) *Phytochemistry* **17**, 1165.

Phytochemistry, Vol. 21, No. 3, pp. 782–784, 1982.
Printed in Great Britain.

0031–9422/82/030782–03$03.00/0

THREE SQUALENE DERIVATIVES FROM *CAULERPA PROLIFERA*

LORENZO DE NAPOLI, ERNESTO FATTORUSSO*, SILVANA MAGNO and LUCIANO MAYOL

Istituto di Chimica Organica e Biologica dell'Università di Napoli, Via Mezzocannone 16, 80134 Napoli, Italy; *Istituto di Chimica Biorganica dell'Università di Napoli, Via L. Rodinò 22, 80138 Napoli, Italy

(*Revised received* 24 *July* 1981)

Key Word Index—*Caulerpa prolifera*; Caulerpaceae; (6*S*, 7*S*)-squalene-6,7-epoxide; (10*S*, 11*S*)-squalene-10, 11-epoxide; all-*trans*-(3*S*)-2,6,10,15,19,23-hexamethyltetracosa-1,6,10,14,18,22-hexaen-3-ol.

Abstract—(6*S*, 7*S*)-Squalene-6,7-epoxide, (10*S*, 11*S*)-squalene-10,11-epoxide and all-*trans*-(3*S*)-2,6,10,15,19,23-hexamethyltetracosa-1,6,10,14,18,22-hexaen-3-ol have been isolated from the marine alga *Caulerpa prolifera*.

Investigations of marine green algae of the family Caulerpaceae have resulted in the isolation of di- and sesquiterpenes [1–4]. From a member of this family, *Caulerpa prolifera*, we recently isolated (*S*)-(−)-squalene-2,3-epoxide (**1**) [5], in addition to two acetylenic sesquiterpenes [1, 2]. This is the first report of the occurrence in nature of **1**, which is known to play an important role in the biogenesis of triterpenes and sterols. We wish to report here that this alga produces, in minor amounts, three structurally related compounds, the epoxides **2**, **3** and the allylic alcohol **4**. Only **3** was previously isolated from a natural source (mycelia of *Sclerotinia fructicola*), but its stereochemistry was not determined [6].

The alga was freeze-dried and the chloroform extracts were purified by Si gel column chromatography. Individual components were then obtained by rechromatography on Si gel and by preparative $AgNO_3$–Si gel TLC.

Compounds **2**, $[\alpha]_D^{20}$ − 11.2°, and **3**, $[\alpha]_D^{20}$ − 3.8°, on