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ISOLATION OF 25(27)-DEHYDROLANOST-8-ENOL FROM *CEREUS GIGANTEUS* AND ITS BIOSYNTHETIC IMPLICATIONS

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Key Word Index—*Cereus giganteus*; Cactaceae; sterols; cycloartenol; 24-methylenecycloartanol; 24-dehydropollinastanol; 24-methylenecholesterol; 25(27)-dehydrolanost-8-enol.

Abstract—From the 4,4-dimethylsterol TLC fraction of the Saquaro cactus pollen cycloartenol, 24-methylenecycloartanol and 25(27)-dehydrolanost-8-enol (a new sterol) were isolated. No pentacyclic triterpenoids were detected at $\geq 0.0001\%$ dry weight. The major sterols isolated from the 4,4-desmethyl sterol band were 24-dehydropollinastanol and 24-methylenecholesterol at 350 mg from 1.0 kg of pollen. The biosynthetic origin of 25(27)-dehydrolanost-8-enol is proposed to be via a pathway involving 2,3:22,23- dioxidosqualene and 24,25-oxidocyloartanol as intermediates.

INTRODUCTION

While it has been known for many years that a 'switching mechanism' operates during seed germination to shunt squalene-oxide from sterol to pentacyclic triterpenoid production [1–3], recently we [4, 5] and others [6–8] have shown that the occurrence of sterol end products is also developmentally regulated throughout the plant life cycle. In our continuing studies on the coordinated changes in lipid composition during plant ontogeny, we describe herein the operation of a sterol pathway and the quiescence of the pentacyclic triterpenoid pathway in cactus pollen. These plants have previously been shown to produce a mixture of sterols (with Δ^5-sterols as the major end products [9]), oxysterols and pentacyclic triterpenoids in mature photosynthetic tissue [9–13] while the pollen is believed to contain a single dominant compound–24-methylenecholesterol [14].

RESULTS AND DISCUSSION

Approximately 5.4 g of a gummy nonsaponifiable lipid fraction (NLF) was obtained after alkaline hydrolysis of an acetone extract of 1.0 kg of pollen grains. The neutral lipids of the NLF were separated into 4,4-dimethyl and 4,4-desmethy sterol fractions by column chromatography (CC) on alumina. The two fractions 700 and 350 mg, respectively were monitored by GLC and the former found to be contaminated with copious amounts of primary long chain fatty alcohols. TLC and reverse phase HPLC were employed to remove the fatty alcohols [15] and to produce sufficient material for spectroscopic analysis (minimally *ca* 100 μg). The TLC purified 4,4-desmethyl sterol CC fraction possessed multiple peaks in GLC between 10 and 30 min (the sterol region). The predominant (*ca* 95% of the material in the 10 to 20 min region) peak was symmetrical and appeared at RR_t of 1.26. GC-MS analysis using a packed 3% SE-30 column of this component indicated an essentially clean sample (M^+ at m/z 398) with the fragmentation pattern of 24-methylenecholesterol. A ^{1}H NMR spectrum obtained on the sample failed to indicate impurities (no significant resonance between δ0.1 and 0.5). However, when the TLC purified sample was injected on to reverse phase HPLC two compounds eluted at α_c of 0.71 and α_c of 0.83 in a ratio of 1:3. The compound at α_c 0.71 was collected, and reinjected into the GLC and found to co-chromatograph with 24-methylenecholesterol. The mass spectra of the two compounds were similar which would account for not detecting the minor component when admixed with 24-methylenecholesterol. The ^{1}H NMR spectrum (Table 1) of the purified unknown was distinguishable from that of 24-methylenecholesterol. The chemical shifts were consistent with the known pollen 4,4-desmethyl-9β,19-cyclosteroid,24-dehydropollinastanol [16, 17]. The similarities in GLC and mass spectral properties of the two compounds probably explains why the earlier investigators failed to detect 24-dehydropollinastanol in cactus pollen [14]. The identification of the other minor 4,4-desmethylsterols was not further pursued.

By GLC analysis the 4,4-dimethylsterol fraction contained a mixture consisting predominantly of primary long chain fatty alcohols (C_{22}–C_{32}) and trace levels of (*ca* 300 μg) cycloartenol, 24-methylenecycloartanol and 25(27)-dehydrolanost-8-enol (lanosta-8,25-dienol). This was subsequently confirmed by mass spectrometry. Adsorption TLC failed to separate the fatty alcohols from the 4,4-dimethylsterols. The adsorption TLC purified sample at R_f 0.50 (which should contain pentacyclic triterpenoids) was spread on to a reversed phase (Whatman C_{18}) TLC plate and developed with methanol–water 19:1). The sterols migrated from the origin to R_f 0.1 while the fatty alcohols remained at the origin [15]. The unsaturated pentacyclic triterpenoids (PT) would have moved in RPTLC with the 4,4-dimethylsterol. Had PT been present their concentration in

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pollen would be $\geq 0.0001\%$ dry wt. GC-MS of the RPTLC purified sample indicated only three compounds and they possessed fragmentation patterns characteristic of sterol intermediates. These sterols were then separated from one another by reverse phase HPLC and their identities determined by their ^1H NMR and mass spectra (see Experimental). Two of the three sterols were the commonly occurring phytosterols–cycloartenol (α_c 1.14) and 24-methylenecycloartanol (α_c 1.28). The third component possessed a GLC RR_t of 1.65 and mass spectrum [M^+ at m/z 426] and ^1H NMR spectrum (Table 1) similar to those of lanosterol. However, the presence of an olefinic proton centred at δ 4.66 in the ^1H NMR spectrum, IR bands at 1647 cm^{-1} (C–C stretch for CH_2) and 883 cm^{-1} (out-of-plane H on a terminal CH_2) due to an unsymmetrically disubstituted ethylenic system, and a base peak at m/z, 55 in the mass spectrum, suggestive of allylic cleavage through the 24,25-bond, indicated the double bond in the side chain was located on the terminal isopropyl group rather than at the 24,25-bond. The spectral values diagnostic for the 25(27)-vinylic system are in agreement with those reported by Blohm *et al* [18] for 25(27)-dehydrocholesterol acetate.

Jain and Gupta claimed to have isolated lanosta-8,25-dienol from the fungus *Fomes fastuous* [19]. Their spectral data, however, do not support its identity, but do support the structure of lupeol, a pentacyclic triterpenoid previously isolated from fungi [20]. We obtained a sample of the 'lanosta-8,25-dienol' from the authors. It cochromatographed on GLC (RR_t 1.80) and HPLC (α_c 0.86) with an authentic sample of lupeol supplied to us by M. J. Thompson (USDA, Beltsville, MD) and the late H. W. Kircher. Additionally the sample had an identical mass spectrum to lupeol (note, the reported diagnositic ions at m/z 218 and 189 given in ref. [19] which are absent from the spectra of compounds with the lanostane skeleton). The ^1H NMR and ^{13}C NMR spectra of the supplied sample were also in accord with the spectra of the lupeol standard. (Also note the signals reported [19] at δ 0.75 and 1.03 and the failure to cite a signal at δ 0.69 for H_3–18).

We propose that the biosynthetic origin of 25(27)-dehydrolanost-8-enol in cactus is developmentally regulated and may involve the 2,3:22,23-dioxidosqualene pathway as shown in Fig. 1. In support of this pathway occurring under physiological conditions is the natural distribution of 25(27)-dehydrocycloartanol [21, 22] and related 25(27)-oxysterol metabolities [23, 24] in tracheophytes and the metabolic conversion of 2,3:22,23-dioxidosqualene to the naturally occuring 24,25-epoxycycloartanol [25] in a cell-free system of *Rubus fruticosa* [26]. The physiological significance of diverting squalene-oxide into dioxidosqualene warrants further study in light of its recognized regulatory importance in mammals [27, 28]. The absence of detectable levels of PT and the preferential occurrence of Δ^5-sterols in pollen may indicate a functional importance for these sterols in reproductive tissues.

EXPERIMENTAL

The pollen of a Saquaro cactus plant was obtained from a pollen monitoring site near Tucson, AZ [26]. The extraction and sterol isolation methods were as previously described [4, 15]. ^1H NMR spectra: 200 MHz. Chemical shifts are expressed in the δ scale downfield from TMS as internal standard. GC-MS was performed as previously described [30] . Direct probe MS was performed at an ion source temp. of 140°. Ultrasphere-ODS C_{18}-column. The eluent was 4% aq. MeOH. The flow rate was 1.6 ml/min; the detector was set at 205nm. HPLC R_t were expressed relative to the α_c for cholesterol (*ca* elution time of 24 min.) GLC was performed on a 3% SE–30 packed column operated at 245° [24]. Retention times are relative to cholesterol, RR_t. Adsorption TLC was developed with benzene–Et_2O (9.1). The 4,4-dimethylsterols possessed R_f 0.5 and the 4,4-desmethylsterols possessed R_f 0.33. With this solvent system long chain fatty alcohols and pentacylic triterpenoids co-chromatograph with 4,4-dimethylsterols, marker compounds were lanosterol, lupeol and triacontanol.

Characterization of sterols. 24-Dehydropollinastanol: TLC, R_f 0.3; GLC, RR_t 1.26; HPLC, α_c 0.71; MS m/z (rel.int): 398 (21), 383 (22), 380 (19), 365 (15), 287 (6), 286 (30, characterizes cyclopropyl group), 269 (6), 267 (3); ^1H NMR (see Table 1). We have shown (Nes *et al.*, P.N.A.S., in press) by NOE and variable temperature studies that the conformation of 24-dehydropollinastanol is pseudoplanar in solution, *vis.*, chair–chair–boat in the A, B, C-rings of the nucleus, respectively, analogous to the solid state [17] rather than having the bent conformation as suggested by Bloch [31].

24-Methylenecholesterol: TLC, R_f 0.30; GLC, RR_t 1.26; HPLC α_c 0.83; MS m/z (rel. int): 398 (20), 383, (14), 380 (10), 365 (7), 314 [100, characterizes 24(28)-methylene group], 273 (4), 255 (5). ^1H NMR (see Table 1).

Table 1. ^1H NMR spectral values for lanosterol and sterols of cactus pollen

Sterol	C-18	C-19	C-21	C-26	C-27	C-28	C-30(α)	C-31(β)	C-32
Lanosterol	0.69(*s*)	1.00(*s*)	0.91(*d*)	1.60(*s*)	1.68(*s*)	—	0.98(*s*)	0.81(*s*)	0.88(*s*)
Lanosta-8, 25-dienol	0.69(*s*)	1.00(*s*)	0.90(*d*)	1.71(*s*)	4.66(*m*)	—	0.98(*s*)	0.81(*s*)	0.88(*s*)
Cycloartenol	0.97(*s*)	$19H_a$-0.56(*d*) $19H_b$-0.33(*d*)	0.87(*d*)	1.61(*br s*)	1.69(*br s*)	—	0.97(*s*)	0.81(*s*)	0.89(*s*)
24-Methylene cycloartanol	0.97(*s*)	$19H_a$-0.56(*d*) $19H_b$-0.35(*d*)	0.90(*d*)	1.03(*d*)	1.03(*d*)	4.48(*d*)	0.97(*s*)	0.81(*s*)	0.90(*s*)
24-Dehydropollinastanol	0.96(*s*)	$19H_a$-0.43(*d*) $19H_b$-0.07(*d*)	0.89(*d*)	1.61(*s*)	1.69(*br s*)	—	—	—	0.89(*s*)
24-Methylene cholesterol	0.68(*s*)	1.01(*s*)	0.91(*d*)	1.03(*d*)	1.03(*d*)	4.68(*d*)	—	—	—

The samples were dissolved in $CDCl_3$ and referenced to TMS: Assignments were confirmed by appropriate decoupling experiments and shift reagents (Eufod) studies using lanosterol, cycloartenol and 24-dehydropollinastanol.

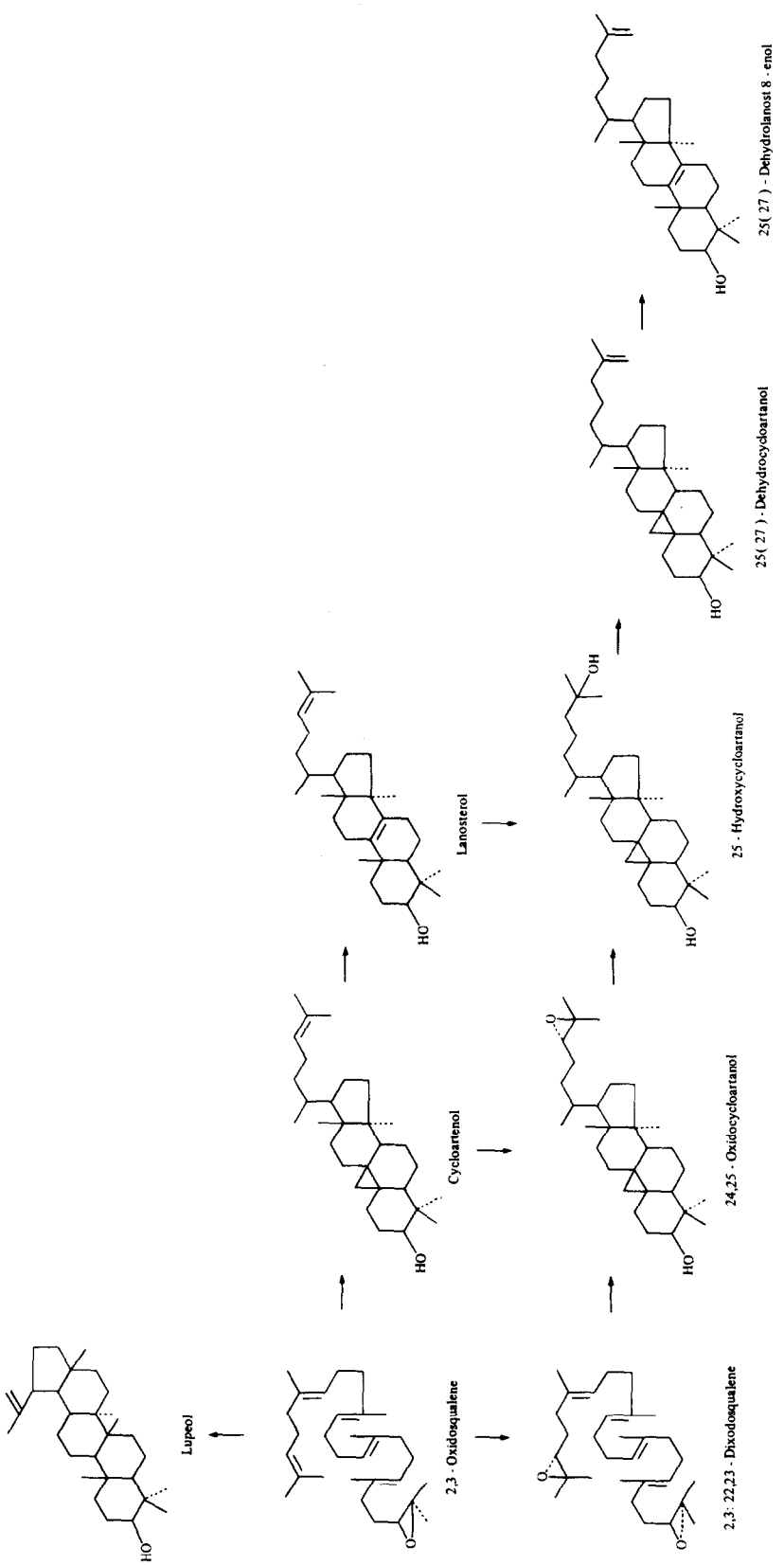

Fig. 1. Proposed pathway for the biosynthesis of 25(27)-dehydrolanost-8-enol in cactus pollen.

Cycloartenol: TLC, R_f 0.50; GLC, RR_t 1.92; HPLC, α_c 1.15; MS *m/z* (rel. int): 426 (17), 411 (13), 408 (23), 393 (20), 365 (11), 286 (19-characterizes cylopropyl group), 271 (6), 259 (6). ^{1}H NMR (see Table 1). 24-Methylenecycloartanol: TLC, R_f 0.50; GLC, RR_t 2.14; HPLC, α_c 1.28; MS *m/z* (rel. int): 440 (17), 425 (15), 422 (15), 407 (25), 315 (5), 300 (33- characterizes cyclopropyl group), 297 (6). ^{1}H NMR (see Table 1).

25(27)-Dehydrolanost-8-enol: TLC R_f 0.50; GLC, RR_t 1.59 HPLC, α_c 1.00, MS *m/z* (rel. int): 426 (37), 411 (76), 393 (32), 271 (5), 255 (6), 241 (7) and significant ions in the low mass region at 109 (56), 95 (67), 81 (55), 69 (80), 55 (100), 43 (39), 41 (69). ^{1}H NMR spectrum (see Table 1); IR, 1647 and 883 cm^{-1}.

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REFERENCES

1. Baisted, D. J., Capstack, E. and Nes, W. R. (1962) *Biochemistry* **1**, 537.
2. Fang, T., and Baisted, D. J. (1975) *Biochem. J.* **150**, 323.
3. Palmer, M. A. and Bowden, B. N. (1977) *Phytochemistry* **16**, 459.
4. Heupel, R. C., Sauvaire, Y., Le, P. H., Parish, E. J. and Nes, W. D. (1986) *Lipids* **21**, 69.
5. Heupel, R. C., Nes, W. D. and Verbeke, J. A. (1987) in *The Metabolism Structure and Function of Plant Lipids* (Stumpf, P. K., Mudd, J. B. and Nes., W. D., eds), p. 53. Plenum Press, New York.
6. Fenner, G. P., Patterson, G. W. and Koines, P. M. (1986) *Lipids* **21**, 49.
7. Garg, V. K. and Nes, W. R. (1985) *Lipids* **20**, 876.
8. Garg, V. K. and Paleg, L. G. (1986) *Physiol Plant* **68**, 335.
9. Salt, T. A., Tocker, J. E., and Adler, J. H. (1987) *Phytochemistry* **26**, 731.
10. Kircher, H. W. and Bird, H. L. (1982) *Phytochemistry* **21**, 1705.
11. Campbell, C. E. and Kircher, H. W. (1980) *Phytochemistry* **19**, 2777.
12. Kircher, H. W. (1980) *Phytochemistry* **19**, 2707.
13. Fonglemann, J. C., Duperrect, S. M. and Kircher, H. W. (1986) *Lipids* **21**, 92.
14. Standifer, L. N., Devys, M. and Barbier, M. (1968) *Phytochemistry* **7**, 1361.
15. Nes, W. D. and Bach, T. J. (1985) *Proc. Royal Soc. Lond. B* **225**, 425.
16. Thompson, M. J., Dutky, S. R., Lehner, Y., Standifer, L. N. and Herbert, E. W. (1978) *Phytochemistry* **17**, 1053.
17. Ducruix, A., Pascard–Billy, C., Derys, M. Barbier, M. and Lederer, E. (1973) *J. Chem. Soc. Chem. Commun.* 929.
18. Blohm, T. R., Stevens, V. L., Kariya, T. and Alig, H. N. (1970) *Biochem Pharmacol.* **19**, 231.
19. Jain, A. C. and Gupta, S. K. (1984) *Phytochemistry* **23**, 2392.
20. Nes, W. D., Heupel, R. C. and Le, P. H. (1984) in *Structure, Function and Metabolism of Plant Lipids* (Sigenthaler, P.-A. and Eichenberger, W., eds), p. 207. Elsevier, New York.
21. Rao, K. L., Ramraj, S. K., Nath, A. R., Rao, T. V. and Sundaramaiah, T. (1986) *Phytochemistry* **25**, 277.
22. Mahato, S. B., Benerjee, S. K. and Chakravarti, R. N. (1971) *Phytochemistry* **10**, 1354.
23. Anjaneyalu, V., Rao, G. S. and Connolly, J. D. (1985) *Phytochemistry* **24**, 1610.
24. Hasegawa, S., Kaneko, N. and Hirose, Y. (1987) *Phytochemistry* **26**, 1095.
25. dePascual Teresa, J., Urones, J. G., Marcos, I. S., Basabe, P., Sexmero, M. J. and Moro, R. F. (1987) *Phytochemistry* **26**, 1767.
26. Heintz, R., Schaefer, P. C. and Benveniste, P. (1979) *J. Chem. Soc. Chem. Commun.* 946.
27. Saucier, S. R., Kandutsch, A. A., Taylor F. R., Spencer, T. A., Phirwa, S. and Gayer, A. K. (1985) *J. Biol. Chem.* **260**, 14571.
28. Panini, S. R., Sexton, R. C., Gupta, A. K., Parish, E. J., Chitrakorn, S. and Rudney, H. (1986) *J. Lipid Res.* **27**, 1190.
29. Schmidt, J. O. and Buchmann, S. L. (1986) *Oecologia (Berlin)* **69**, 491.
30. Nes, W. D. and Stafford, A. E. (1983) *Proc. Natl Acad. Sci.* **80**, 3227.
31. Bloch, K. E. (1983) *CRC Crit. Rev. Biochem.* **14**, 47.