

Structural Elucidation of a New Group of Secostictane Triterpenoids

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By a combination of spectroscopic and chromatographic procedures it was established that three new triterpenoids isolated from the extractives of the lichen *Pseudocyphellaria degelii* possessed the novel 3,22-disubstituted 3,4-secostict-4(23)-ene structures (2a), (2e), and (2h) respectively.

DURING the course of a recent chemotaxonomic survey of the genus *Pseudocyphellaria* (lichens) in New Zealand it was noted¹ that in Herbaria specimens of *P. degelii* D. Gall. *ined.*, there occurred variable quantities of four triterpenoid substances, three of which had not hitherto been structurally investigated. Nine of the nineteen specimens examined contained variable (often trace) quantities of the known² stictane-3 β ,22 α -diol (1a) together with a substance (pd-1) of lower R_F value. A

further eight specimens contained pd-1, the diol (1a) and a substance (pd-2) of higher R_F value, whilst another two specimens contained the diol (1a), pd-2, and an additional higher R_F value substance (pd-3). The three foregoing lichen populations were designated as the α -, β -, and γ -forms respectively. Since pd-1, pd-2, and pd-3 appeared to be new substances efforts were made to secure them in quantities sufficient for structural analysis and, although only modest quantities of the

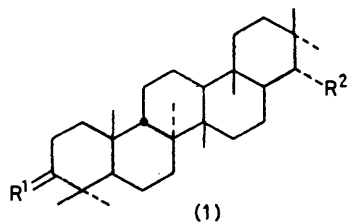
¹ D. J. Galloway, P. W. James, and A. L. Wilkins, unpublished results.

² W. J. Chin, R. E. Corbett, C. K. Heng, and A. L. Wilkins, *J.C.S. Perkin I*, 1973, 1437.

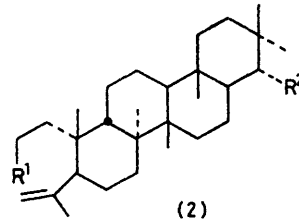
lichen materials (mainly the α -form) could be procured for extraction, it proved possible by a combination of spectroscopic and chromatographic procedures to identify each of the substances.

From the extractives of the α -form lichen material there was isolated an unsaturated acidic triterpene alcohol (pd-1), $C_{30}H_{50}O_3$, ν_{max} . (Nujol) 3 430 (OH),

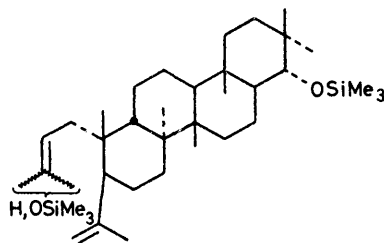
(KBr) 1 736 and 1 242 cm^{-1} (OCOCH₃). In the 1H n.m.r. spectrum of pd-1-methyl ester there appeared six tertiary methyl group signals (see Table 1), an olefinic methyl group signal (δ 1.78, $W_{\frac{1}{2}}$ 2.5 Hz) and a two-proton olefinic methylene signal (δ 4.88, $W_{\frac{1}{2}}$ 6.5 Hz) together with a one proton doublet (δ 3.15, $CHOH$, J 10 Hz) and a methoxycarbonyl group signal at δ 3.65.



- a; $R^1 = H$, $\beta - OH$; $R^2 = OH$
 b; $R^1 = H$, $\beta - OAc$; $R^2 = OH$
 c; $R^1 = O$; $R^2 = OH$
 d; $R^1 = H_2$; $R^2 = OH$
 e; $R^1 = H_2$; $R^2 = OAc$
 f; $R^1 = H$, $\beta - OSiMe_3$; $R^2 = OSiMe_3$
 g; $R^1 = H$, $\beta - OAc$; $R^2 = OSiMe_3$
 h; $R^1 = O$; $R^2 = OSiMe_3$



- a; $R^1 = CO_2H$; $R^2 = OH$
 b; $R^1 = CO_2Me$; $R^2 = OH$
 c; $R^1 = CO_2Me$; $R^2 = OAc$
 d; $R^1 = CO_2SiMe_3$; $R^2 = OSiMe_3$
 e; $R^1 = CHO$; $R^2 = OH$
 f; $R^1 = CHO$; $R^2 = OSiMe_3$
 g; $R^1 = CH_2OH$; $R^2 = OH$
 h; $R^1 = CH_2OAc$; $R^2 = OH$
 i; $R^1 = CH_2OAc$; $R^2 = OAc$
 j; $R^1 = CH_2OAc$; $R^2 = OSiMe_3$



3 200—2 600, 1 705 (CO_2H), 1 635, and 890 cm^{-1} ($CH_2=C$). Methylation afforded a methyl ester (pd-1-methyl ester), $C_{31}H_{52}O_3$, ν_{max} . (Nujol) 1 735 and 1 170

TABLE 1

Assignment of 1H n.m.r. methyl group signals (δ in $CDCl_3$) appearing in some stictane and 3,4-secostict-4(23)-ene triterpenoids

| Substance | 4 α | 4 β | 10 β | 8 α | 14 β | 18 β | 21 α | 21 β |
|-----------|------------|-----------|------------|------------|------------|------------|-------------|------------|
| (1d) | 0.86 | 0.81 | 0.91 | 1.14 | 0.90 | 0.73 | 0.86 | 0.98 |
| (1e) | 0.85 | 0.81 | 0.89 | 1.14 | 0.92 | 0.82 | 0.85 | 0.92 |
| (2b) | | | 1.05 | 1.05 | 0.87 | 0.76 | 0.84 | 0.98 |
| (2c) | | | 1.02 | 1.07 | 0.84 | 0.84 | 0.84 | 0.93 |
| (2e) | | | 1.03 | 1.06 | 0.87 | 0.78 | 0.87 | 0.99 |
| (2g) | | | 1.04 | 1.04 | 0.87 | 0.76 | 0.87 | 0.98 |
| (2h) | | | 1.03 | 1.03 | 0.87 | 0.73 | 0.84 | 0.98 |
| (2j) | | | 1.03 | 1.03 | 0.84 | 0.84 | 0.84 | 0.93 |

cm^{-1} (CO_2CH_3), which on subsequent acetylation yielded an acetate (pd-1-methyl ester-acetate), $C_{33}H_{54}O_4$, ν_{max} .

That the olefinic methyl and methylene group signals were mutually coupled across a common double bond was established when irradiation of the former sharpened the latter's signal, and *vice versa*; hence the presence of an isopropenyl group was established.

The presence in pd-1, and its derivatives of an isopropenyl group, together with six tertiary methyl groups and a methoxycarbonyl group (a total of nine terminal carbon residues) indicated that the foregoing substances possessed a novel molecular framework, since in the majority of tetracyclic and/or pentacyclic triterpenoids derived from squalene precursors there characteristically occurs, as in squalene itself, a maximum of eight such terminal carbon (usual methyl group) residues. The requirement for a ninth such residue can, however, be fulfilled by a seco-pentacyclic triterpenoid skeleton, in which ring cleavage has occurred adjacent to an initially fully substituted carbon centre, the additional terminal carbon residue (for example a carboxy-group) being associated with a seco-carbon atom. The molecular

formulations indicated earlier for pd-1 and its derivatives were also in accord with the conclusion that these substances possessed an isopropenyl substituted tetracyclic skeleton. That pd-1, its methyl ester, and derived acetate possessed the 3,22-disubstituted 3,4-secostict-4(23)-ene structures (2a), (2b), and (2c) respectively was deduced from the following ^1H n.m.r. and mass spectral evidence.

In the ^1H n.m.r. spectrum of pd-1 methyl ester (2b) and its acetate (2c) there appeared methine doublets (J 10 Hz) centred at δ 3.15 and 4.72 respectively reminiscent of those appearing in the spectra of 22 α -hydroxy and acetoxy substituted stictane triterpenoids.² A similar correspondence (see Table 1) was also apparent in the resonance frequencies of the three ring E methyl group signals, but not in the C-8, C-10, and C-14 methyl group signals. This apparent discrepancy is, however, in accord with the boat ring B structure advanced for the parent group of stictane triterpenoids, since in 3,4-secostictane triterpenoids it is to be anticipated that

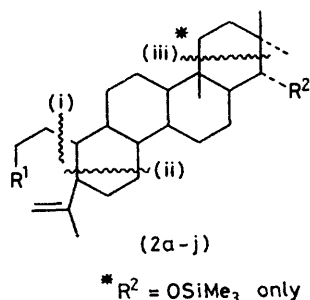


FIGURE Mass spectral cleavages of rings A, B, and E occurring in some 3,4-secostictane triterpenoids.

the rupture of ring A could allow ring B to revert to an alternative twist boat, or twist chair, conformation.

The origin and structural significance of the principal fragments appearing in the electron-impact mass spectra of C-2, C-3, and/or C-22 substituted stictane triterpenoids have recently been reported.³ Without exception the fragments which in the parent group of 22 α -substituted stictane triterpenoids were established to involve ruptures of rings C, D, and/or E could also be located in the new group of seco-triterpenoids. One of these fragments, an intense $M^+ - 71$ fragment [cleavage (iii), Figure] occurring only in 22 α -trimethylsilyl derivatives is considered³ to characterize uniquely the Wagner-Meerwein rearranged ring E structure which occurs in stictane triterpenoids. By contrast, however, the ring A/B fragmentation patterns of the respective groups of tetracyclic and pentacyclic triterpenoids differed markedly. Two cleavages which occur in pd-1 and its derivatives, but not the parent group of pentacyclic triterpenoids, support the 3,4-seco-ring A structures advanced for the new group of triterpenoids.

* For details of the Supplementary publications scheme, see Notice to Authors No. 7, in *J.C.S. Perkin I*, 1977, Index Issue.

² P. T. Holland and A. L. Wilkins, *Org. Mass Spectroscopy*, 1978, in print.

Substituent group mass shifts indicated fission of the C-1/C-10 bond to proceed with the loss of a terminally substituted three-carbon unit [cleavage (i), Figure 1], whilst the loss of a C_6H_9 radical can be considered to arise by cleavage across ring B [cleavage (ii), Figure 1]. Further details of these mass spectral correlations, and supporting high resolution data appear in the Supplementary Publication No. SUP 22350 (4 pp).*

In addition to quantities of the diol (1a) and the acid (2a) there was also isolated from the extractives of the β -form lichen material an aldehydic substance (pd-2), $\text{C}_{30}\text{H}_{50}\text{O}_2$, the ^1H n.m.r. and mass spectral features of which indicated it to be the aldehydic equivalent of the acid (2a). In accord with the assignment of structure (2e) to this compound, it yielded on reduction with lithium aluminium hydride a seco-diol (2g) identical to that also prepared by the similar reduction of pd-1-methyl ester (2b).

Although less than a gram of the γ -form lichen material was available for extraction it proved possible to identify the substances present by use of the combined gas chromatography-mass spectroscopy (g.c.-m.s.) technique. After a preliminary separation of the extracted triterpenoids on a small silica-gel plate, portions of the three triterpenoid fractions so obtained were treated with trimethylsilylimidazole in pyridine prior to g.c.-m.s. analysis. Thus it was established that the additional substance occurring in the higher R_F fraction was the hydroxy-acetate (2h), for the mass spectrum of the silylated derivative (2j) showed $M^+ - 71(\text{C}_5\text{H}_{11})$ and $M^+ - 101(\text{C}_5\text{H}_9\text{O}_2)$ fragments indicating the presence of a 22 α -trimethylsilyl group and a C-3 acetoxy-group respectively (see Figure 1). Confirmation of these structural assignments was obtained when partial acetylation of the seco-diol (2g) afforded a monoacetate identical (by g.c.-m.s. analysis) with the lichen substance. Similarly it was demonstrated by g.c.-m.s. analysis that the intermediate fraction consisted largely of the aldehyde (2e) and that 3 β -acetoxy-stictan-22 α -ol (1b) and 22 α -hydroxystictan-3-one (1c) were minor constituents of the upper and intermediate fractions respectively. Only the diol (1a) was detected in the lower fraction.

The g.c.-m.s. behaviour of the aldehyde (2e) deserves further comment, for depending on the silylation conditions, and sample age, variously between one and four peaks were observed in the gas chromatograms of this substance. For example, mild silylation with bis-(trimethylsilyl)trifluoroacetamide in pyridine (25 $^\circ\text{C}$, 30 min) of a freshly isolated sample yielded essentially a single peak (m/e 514, M^+ for $\text{C}_{33}\text{H}_{58}\text{O}_2\text{Si}$), whereas chromatograms of an aged sample invariably possessed an additional peak due to the presence of appreciable quantities of the silylated acid (2d) (m/e 602, M^+ for $\text{C}_{36}\text{H}_{66}\text{O}_3\text{Si}_2$). However when the aldehyde was silylated

⁴ (a) G. R. Chopra, A. C. Jain, and T. R. Seshadri, *Indian J. Chem.*, 1969, **7**, 1179; (b) M. M. Rao, H. Meshulam, R. Zelnik, and D. Lavie, *Tetrahedron*, 1975, **31**, 333.

⁵ G. H. Whitham, *J. Chem. Soc.*, 1960, 2016.

under more forcing conditions with trimethylsilylimidazole in pyridine (100 °C, 60 min) two new substances were obtained, the mass spectral features of which revealed them to be the *cis*- and *trans*-enol-trimethylsilyl esters, (3a) and (3b) (*m/e* 586, M^+ for $C_{36}H_{66}O_2Si_2$).

The structures advanced for the new group of seco-stictane triterpenoids are not without biological precedent, similarly substituted groups of 3,4-secofriedelane,⁴ and 3,4-seco-olean-12-ene,⁵ 3,4-secourane,⁶ and 3,4-secolupane⁷ being triterpenoids known.

EXPERIMENTAL

General directions are as in a related publication. E—H refers to ether—hexane solvent combinations. Mass spectral data for compounds (2a), (2b), (2c), (2d), (2e), (2f), (2h), and (2j) appear in the Supplementary Publication No. SUP 22350 (4 pp).^{*} Combined (g.c.—m.s.) analyses were conducted as described in ref. 3.

Isolation of Triterpenoids.—(a) The finely ground α -form lichen material (14.4 g), collected from the shores of Lake Waikareiti in January 1976, was extracted with chloroform in a Soxhlet apparatus for 12 h. Evaporation of the solvent gave a gummy residue (880 mg) which was separated by p.l.c. on silica gel with E—H (1 : 1) into four fractions. T.l.c. indicated that the two fractions with higher R_F value (combined weight 210 mg) consisted largely of a single metabolite (pd-1), whilst the two fractions with lower R_F value were shown to consist largely of yellow pigments and were not further investigated. Crystallization of a portion of the materials with higher R_F value gave 22 α -hydroxy-3,4-secostict-4(23)-en-3-oic acid (2a) (20 mg), m.p. 235—237 °C (from acetone), ν_{max} (Nujol) 3 430 (OH), 3 200—2 600, 1 705 (CO₂H), 1 635, and 890 cm⁻¹ (C=CH₂); *m/e* 458 (M^+) (Found: C, 78.8; H, 10.8. $C_{30}H_{50}O_3$ requires C, 78.6; H, 10.9%).

(b) The finely ground β -form lichen material (3 g), collected in the vicinity of the Homer Tunnel, Fiordland, in August 1976, was extracted as in (a) above. Separation of the extractives (215 mg) by multiple ($\times 3$) p.l.c. on silica gel with E—H (1 : 2) gave in order of decreasing R_F value, the aldehyde (2e) (24 mg), the diol (1a) (20 mg), m.p. and mixed α m.p. 283—284 °C, and the acid (2a) (18 mg).

22 α -Hydroxy-3,4-secostict-4(23)-en-3-al (2e), an oil, had ν_{max} (Nujol) 3 450 (OH), 1 700 (CHO), 1 635, and 888 cm⁻¹ (C=CH₂); δ 1.80 (3 H, $W_{\frac{1}{2}}$ 3 Hz, C=C—CH₃), 3.15 (1 H, d, J 10 Hz, CHOH), 4.82 (2 H, $W_{\frac{1}{2}}$ 7 Hz, C=CH₂), and 9.75 (1 H, $W_{\frac{1}{2}}$ 4 Hz, CHO); *m/e* 442 (M^+). G.c.—m.s. results indicated the aldehyde (2e) was oxidized to the acid (2a) when set aside in the air.

(c) The finely ground γ -form lichen material (0.9 g), collected in the vicinity of Swampy Summit, Dunedin, in February 1952, was extracted as in (a) above. Separation as in (b) above afforded the diol (1a) (8 mg) (lower band), and two fractions of higher R_F value, (4 mg) (upper band) and (5 mg) (intermediate band).

Small portions of the foregoing three fractions were treated with trimethylsilylimidazole (see ref. 5) and/or bis(trimethylsilyl)trifluoroacetamide (see text) in pyridine (1 : 1) (150 μ l) prior to g.c.—m.s. analysis on OV-101 and/or OV-17 columns. Comparative g.c.—m.s. analysis of silyl-

ated standards, and also the substances isolated in (a) and (b) above, established the major constituents of the upper and intermediate fractions to be the monoacetate (2h) and the aldehyde (2e), whilst 3 β -acetoxystictan-22 α -ol² (1b) and 22 α -hydroxystictan-3-one² (1c) were demonstrated to be minor constituents of the respective fractions, and only the diol (1a) was detected in the fraction of lowest R_F value. The retention index data for the silylated derivatives (1f), (1g), (1h), (2d), (2f), and (2j) appear in Table 2.

TABLE 2

Methylene unit retention indexes for some stictane and 3,4-secostictane triterpenoids

| Compound | Column | |
|----------|--------|-------|
| | OV-101 | OV-17 |
| (2d) | 36.2 | 37.85 |
| (2f) | 35.8 | 38.4 |
| (2j) | 35.9 | 38.1 |
| (1f) | 36.1 | 37.4 |
| (1g) | 36.45 | 38.4 |
| (1h) | 35.4 | 38.3 |

cis- and *trans*-Enol-trimethylsilyl Esters (3a,3b).—A solution of the aldehyde (2e) in trimethylsilylimidazole—pyridine (1 : 1) (150 μ l) was heated, with occasional shaking, for 1 h at 100 °C. G.c.—m.s. analysis on an OV-17 column indicated the presence in the reaction mixture of the *cis*- and *trans*-enol-trimethylsilyl esters (3a) and (3b) in a ca. 1 : 1 ratio.

Enol-ester-1 (the more-volatile constituent) (r.r.i. 36.15 m.u.) had *m/e* 586 (M^+ , 4%), 571 (8), 496 (10), 481 (12), 457 (59), 367 (22), 319 (31), 202 (95), 200 (82), and 191 (100).

Enol-ester-2 (the less-volatile constituent) (r.r.i. 37.4 m.u.) had *m/e* 586 (M^+ , 8%), 571 (5), 496 (10), 481 (10), 457 (36), 367 (13), 319 (19), 202 (100), 200 (86), and 191 (82).

Methyl 22 α -Hydroxy-3,4-secostict-4(23)-en-3-oate (2b).—A solution of the acid (2a) (160 mg) in ether (70 ml) was stirred with an excess of diazomethane for 8 h at 20 °C. Work-up gave a residue (165 mg) which was taken up in benzene (30 ml) and chromatographed on alumina. Elution with E—H (1 : 1) gave *methyl 22 α -hydroxy-3,4-secostict-4(23)-en-3-oate* (2b) (146 mg), m.p. 115—118 °C (from acetone), ν_{max} (Nujol) 3 580—3 450 (OH), 1 735 (CO₂CH₃), 1 632, and 980 cm⁻¹ (C=CH₂); δ 1.78 (3 H, $W_{\frac{1}{2}}$ 3 Hz, C=C—CH₃), 3.15 (1 H, d, J 10 Hz, CHOH), 3.65 (3 H, CO₂CH₃), and 4.88 (2 H, $W_{\frac{1}{2}}$ 7 Hz, C=CH₂) (Found: *m/e* 472.389 2. $C_{31}H_{52}O_3$ requires *m/e* 472.391 9).

Methyl 22 α -Acetoxy-3,4-secostict-4(23)-en-3-oate (2c).—A solution of the methyl ester (2b) (40 mg) in pyridine—acetic anhydride (1 : 1) was stirred for 24 h at 20 °C. Work-up and purification by p.l.c. on silica gel with E—H (1 : 2) gave *methyl-22 α -acetoxy-3,4-secostict-4(23)-en-3-oate* (2c) (36 mg), m.p. 128—130 °C (from acetone), ν_{max} (KBr) 1 736, 1 242 (CO₂CH₃ and OCOCH₃), 3 068, 1 640, 885, and 889 cm⁻¹ (C=CH₂); δ 1.78 (3 H, $W_{\frac{1}{2}}$ 3 Hz, C=C—CH₃), 2.05 (3 H, OCOCH₃), 3.67 (3 H, CO₂CH₃), 4.72 (1 H, d, J 10 Hz, CHOCOCH₃), and 4.87 (2 H, $W_{\frac{1}{2}}$ 7 Hz, C=CH₂); *m/e* 514 (M^+) (Found: C, 77.2; H, 10.3. $C_{33}H_{54}O_4$ requires C, 77.0; H, 10.5%).

3,4-Seconstict-4(23)-ene-3,22 α -diol (2g).—(a) A solution of the methyl ester (2b) (38 mg) in ether (70 ml) was heated under reflux with an excess of lithium aluminium hydride for 1 h. Work-up and purification by p.l.c. on silica gel

* See footnote on p. 1562.

⁶ L. Mangoni and M. Belardinii, *Tetrahedron Letters*, 1963, 14, 921.

⁷ R. M. Carman and D. Cowley, *Austral. J. Chem.*, 1965, 18, 213.

with E—H (1 : 1) gave 3,4-secostict-4(23)-ene-3,22 α -diol (2g) (22 mg), m.p. 88—90 °C (sublimed sample), ν_{\max} (Nujol) 3 540—3 400 (OH), 1 635, and 890 cm^{-1} (C=CH₂); δ 1.78 (3 H, $W_{\frac{1}{2}}$ 3 Hz, C=C—CH₃), 3.14 (1 H, d, J 10 Hz, CHOH), 3.60 (2 H, $W_{\frac{1}{2}}$ 12 Hz, CH₂OH), and, 4.79 (2 H, $W_{\frac{1}{2}}$ 7 Hz, C=CH₂); m/e 444 (M^+ , 16%), 426 (25), 411 (19), 385 (65), 363 (20), 345 (45), 207 (88), and 189 (100) (Found: C, 81.2; H, 12.0. C₃₀H₅₂O₂ requires C, 81.0; H, 11.8%).

(b) Similar reduction of the aldehyde (2e) (25 mg) gave the diol (2g) (21 mg).

3-Acetoxy-3,4-secostict-4(23)-en-22 α -ol (2h).—A solution of the diol (2g) (36 mg) and pyridine-acetic anhydride (29 : 1) (15 ml) was stirred for 2 h at 20 °C. Work-up and purification by p.l.c. on silica gel with E—H (1 : 4) gave 3-acetoxy-3,4-secostict-4(23)-en-22 α -ol (2h) (32 mg), m.p. 114—116 °C (sublimed sample), ν_{\max} (Nujol) 3 450 (OH), 1 735, 1 245 (OCOCH₃), 1 635, and 888 cm^{-1} (C=CH₂); δ 1.78 (3 H, $W_{\frac{1}{2}}$ 3.5 Hz, C=C—CH₃), 2.03 (3 H, OCOCH₃), 3.15 (1 H, d, J 10 Hz, CHOH), 3.98 (2 H, $W_{\frac{1}{2}}$ 10 Hz, CH₂OCOCH₃), and 4.82 (2 H, $W_{\frac{1}{2}}$ 7 Hz, C=CH₂); m/e 486 (M^+) (Found: C, 79.2; H, 10.9. C₃₂H₅₄O₃ requires C, 79.0; H, 11.2%).

3,22 α -Diacetoxy-3,4-secostict-4(23)-ene (2i).—A solution of

the diol (2g) (18 mg) in pyridine-acetic anhydride (1 : 1) (10 ml) was stirred for 24 h at 20 °C. Work-up and purification by p.l.c. on silica gel with E—H (1 : 4) gave 3,22 α -diacetoxy-3,4-secostict-4(23)-ene (2i) (16 mg), m.p. 131—133 °C (sublimed sample), ν_{\max} (Nujol) 1 735, 1 245 (OCOCH₃), 1 638, and 890 (C=CH₂) cm^{-1} ; δ 1.78 (3 H, $W_{\frac{1}{2}}$ 3.5 Hz, C=C—CH₃), 2.05 (6 H, 2 \times OCOCH₃), 3.97 (2 H, $W_{\frac{1}{2}}$ 12 Hz, CH₂OCOCH₃), 4.70 (1 H, d, J 10 Hz, CHOCOCH₃), and 4.83 (2 H, $W_{\frac{1}{2}}$ 7 Hz, C=CH₂); m/e 528 (M^+ , 11%), 468 (40), 453 (14), 427 (94), 425 (13), 387 (76), 367 (21), and 189 (100) (Found: C, 77.4; H, 10.5. C₃₄H₅₆O₄ requires C, 77.2; H, 10.7%).

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