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CHEMICAL INVESTIGATION OF VOLATILE CONSTITUENTS OF *INULA VISCOSA* AIT

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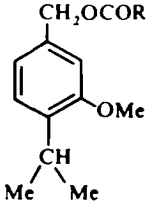
Abstract—A novel monoterpene of the oxygenated thymol type, namely *p*-cymene-3-methoxy-7-ol (IV) was isolated from the young roots of *Inula viscosa* Ait, as its isobutyrate (II) and isovalerate (I). A third volatile component accompanying I and II was nerylisovalerate (III). The structures of I, II and III were determined by IR, NMR and mass spectroscopy and confirmed by synthesis.

INVESTIGATION of the chemical constituents of the roots of *Inula viscosa* Ait revealed a marked difference between young and old roots. Light petroleum extracts of young roots yielded an oily fraction which gave an oil constituting about 1% of their total dry weight. Old roots, however, contained only traces of this volatile oily fraction. The IR spectrum of the volatile oily fraction showed strong aromatic absorptions. On TLC, this fraction gave one tailing spot which could not be separated by column chromatography. On PVC, the distillate was found to contain at least 10 components of which three appeared in major proportions. These three compounds (I, II and III) appeared in *ca* 50%, 30% and 10%, respectively. The remaining volatile constituents, which had the shortest retention-time due to their relatively low molecular weight, were not separated. Compounds I, II and III were separated by preparative gas-chromatography in a high state of purity (>95%). Compound II, having the empirical formula $C_{15}H_{22}O_3$, is an aromatic ester as deduced from its IR absorption spectrum (1730, 1250, 1605, 1575, 1500, 845, 810 cm^{-1}). The NMR spectrum of II [δ 6·08d (J = 1·5 c/s) (1H); δ 6·88dd (J = 8; 1·5 c/s) (1H); δ 7·17d (J = 8 c/s) (1H) (aromatic protons); δ 1·19d (J = 7 c/s) (12H), δ 2·60 h (J = 7 c/s) (1H) and δ 3·30* h (J = 7 c/s) (1H) (two isopropyl groups); δ 3·81 s (3H) (Me) and δ 5·07 s (2H) (two benzylic protons)], is consistent with the proposed structure of 3-methoxy-4-isopropylbenzyl-isobutyrate, a novel substituted monoterpenic structure of the oxygenated thymol type.



I: R = —CH_2CHMe_2
II: R = —$CHMe_2$

* A shift of −0·4 ppm in comparison with the cumene's i-Pr group is readily explained by the influence of an unpaired electron-pair on the O atom of a neighbouring *o*-methoxy substituent.

Alkaline hydrolysis of II gave isobutyric acid and the new benzyl alcohol derivative 3-methoxy-4-isopropylbenzylalcohol (IV). Cleavage of the ester bond of II, yielding IV, was also invariably affected as a result of prolonged retention of II on an alumina column. The synthesis of IV was accomplished according to Scheme 1, starting with 3-hydroxycumic acid.[1]

The NMR spectrum of the synthetic 3-methoxy-4-isopropylbenzylalcohol (IV) showed the same characteristic pattern of 1,2,4-trisubstituted benzene ring previously described for II [δ 7·22d (J = 8 c/s) (1H); δ 6·80dd (J = 8; 1·5 c/s) (1H); δ 6·81d (J = 1·5 c/s) (1H)]. Its other signals at δ 3·74s (3H) (—OMe); δ 3·28 h (J = 7 c/s) (1H); and δ 1·19 d (J = 7 c/s) (6H) (—$CH(Me_2)$; also closely compared with those of II, with the only exception in the value of the benzylic protons' signal being shifted by 0·5 ppm to a higher field as expected, following hydrolysis of the ester [δ 4·50 s (2H)]. Treatment of IV with isobutyryl chloride gave II—identical in every respect with the naturally occurring one.

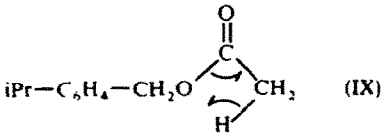
A similar oxygenated thymolic structure in which the two alcoholic functions are esterified, was recently proposed for an inseparable mixture of two natural compounds, on the basis of their NMR data.[2] To our knowledge, this is the only other substituted oxygenated thymol reported.

Substance I, having the empirical formula $C_{16}H_{24}O_3$, exhibited an IR absorption spectrum very similar to that of II. The mass spectrum of I (*vide infra*), as well as its NMR [δ 7·17d (J = 8), δ 6·89dd (J = 8; 1·5) and δ 6·81d (J = 1·5) aromatic protons; δ 3·80 s (OMe), δ 1·20d (J = 7) and δ 3·30 h (J = 7) i-Pr; δ 5·07 s —CH_2OR; δ 1·95–2·40 m and δ 0·95 m i-valerate protons], clearly indicated that I was the next higher homologue of II. Comparison of the NMR spectra of I with those of the various isomers of valeric acid, unequivocally established the structure of the acidic component of I as that of isovaleric acid. The almost equal chemical shift of both the isopropyl proton and the two protons α to the carboxyl group, resulted in the observed second-order spectrum of isovaleric acid. Even in such a second-order spectrum, the appearance of a multiplet corresponding to the two Me groups of isovaleric acid is quite unusual. Alkaline hydrolysis of I in methanolic KOH yielded, as expected, isovaleric acid and alcohol IV, the latter upon treatment with isovaleryl chloride regenerated the ester I; hence, the structure of I is 3-methoxy-4-isopropylbenzylisovalerate.

Mass spectra. In order to interpret the mass spectra of compounds I and II, the fragmentation pattern of their corresponding alcohol (IV) was first analysed. The rationalisation of the mass spectral fragmentation of IV (Scheme 2), is based on the metastable peaks as well as on the appropriate shifts in the corresponding fragments and metastable peaks of the deuterated compounds d_2-IV and d_3-IV (prepared as shown in Scheme 1). Alcohol IV clearly exhibits the typical fragmentation behaviour expected from benzyl alcohols and alkyl anisoles.[3] The sequential loss of three Me radicals in IV giving rise to *m/e* 165, 150 and 135 fragments, should be noted since it is quite uncommon; the same process is also evident in a structurally related compound 2-isopropyl-5-methylanisole (Scheme 3). Also worthy of mention are the series of double charged fragments arising both from the alcohol IV (83·5, 82·5, 81·5, 80·5, 76·5, 75·5, 74·5, 69·5, 68·5, 60·5), and from 2-isopropyl-5-methylanisole (74·5, 73·5, 72·5, 68·5, 67·5, 66·5, 65·5, 57·5, 51·5).

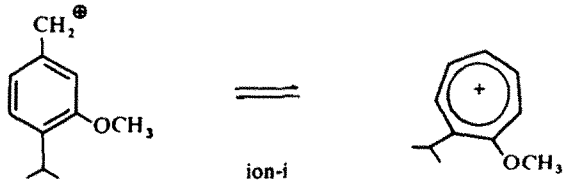
Having analysed the spectrum of IV, the fragmentation patterns of its various

esters (I; II; d_2-II; IX, d_3-IX) are quite clear. In each of these spectra, the strongest peak observed was M-15 (100%), whereas the molecular peak was only second in intensity. In all of the esters, ketene elimination as described below for the acetate, IX, gave rise to alcohol IV, which further fragmented as depicted in Scheme 2.



(IX)

In addition to the fragments arising from the alcohol (IV), the ester function gave rise also to the following fragments: *m/e* 163 (10%) corresponding to ion i, from



which two further fragments may arise: *m/e* 148 (10%) (163-CH_3) and *m/e* 133 (7%) (163-CH_2O or 163-C_2H_6), and a second series derived from the expected acylium ions (*m/e* 85 (20%), *m/e* 57 (31%) and *m/e* 43 (33%) for I, and *m/e* 71 (22%) and *m/e* 43 (46%) for II). These fragmentation patterns are very characteristic and lend themselves to the ready identification of the acid components of the esters.

Substance III has a molecular weight close to those of I and II (M^+ 238), and its empirical formula is $C_{15}H_{26}O_2$. The IR spectrum of III showed typical absorptions of an ester group (1, 730, 1250 cm^{-1}), however, the aromatic absorptions seen in the IR spectra of I and II were absent. The NMR spectrum of III showed a signal at δ 0·94 (6H) which, upon broadening, gave exactly the same characteristic pattern as that of the Me groups of isovaleric acid present in I. It appeared, therefore, that III was another isovaleric acid ester. If correct, then as found, the three remaining protons of the isovaleric component of III resonate in the δ 1·85–2·20 region, where the signals of four additional allylic protons appear. The other signals in the NMR spectrum of III were interpreted as follows: δ 1·60 s (3H); δ 1·68 s (3H); δ 1·75 s (3H)—3 Me groups attached to olefinic bonds; δ 5·06 m (1H) and δ 5·28 t (J = 7 c/s) (1H)—two vinylic protons; δ 4·47 d (J = 7 c/s) (2H)—two protons attached to the OH-bearing C atom. The correlation between the doublet at δ 4·47 and the triplet at δ 5·28 was confirmed by a spin-spin decoupling experiment, whereby irradiation of each of the signals changed the other one into a singlet. The shape of the signals in the lower region of the field, was identical to those displayed by the corresponding protons of geranyl or neryl-acetate. The spectral data suggested, therefore, the structure of geranyl- or neryl-isovalerate for compound III. In order to distinguish between the two isomers, the NMR spectra of geraniol, nerol and their acetates were recorded

(Table 1). The data indicates that whereas in nerol or its acetate the three Me groups appear separately, in geraniol and its ester two of the Me groups overlap. Therefore, taking into account the NMR spectrum of III, it should be neryl-isovalerate, as confirmed by synthesis.

TABLE 1

Compound	$(CH_3)_2C...CH-$	$CH_3-\underset{\vert}{C}=CH-CH_2OR$	
Geraniol	1·62	1·68	1·68
Nerol	1·60	1·68	1·74
Geranyl-acetate	1·61	1·70	1·70
Neryl-acetate	1·61	1·68	1·76

The mass spectrum of III showed the expected fragmentation pattern of neryl esters giving rise to two series of peaks; one originating in the nerol, the other arising from the acid itself.[4] The molecular peak of III appeared at *m/e* 238 (4%) accompanied by the elimination product of valeric acid at *m/e* 136 [M-102] (27%), (the base peak being *m/e* 69 [$Me_2C{=}CHCH_2^{\oplus}$]). This elimination product is, in fact, the olefin obtained also from geraniol[4] giving rise to similar fragmentations: *m/e* 121 [M-(102 + 15)] (32%) (loss of methyl), and *m/e* 93 [M-(102 + 43)] (76%) (loss of isopropyl). The expulsions of 15 and 43 mass units, are the most favourable process in monoterpenic hydrocarbons and in hydrocarbonic fragments derived from monoterpenic alcohols.[4] Cleavage of the bond adjacent to the ester group, gives rise to a second series of peaks as follows: *m/e* 85 [$Me_2CHCH_2C{\equiv}O^{\oplus}$] (43%) and *m/e* 57 (85-CO) [$(CH_3)_2CHCH_2^{\oplus}$] (52%).

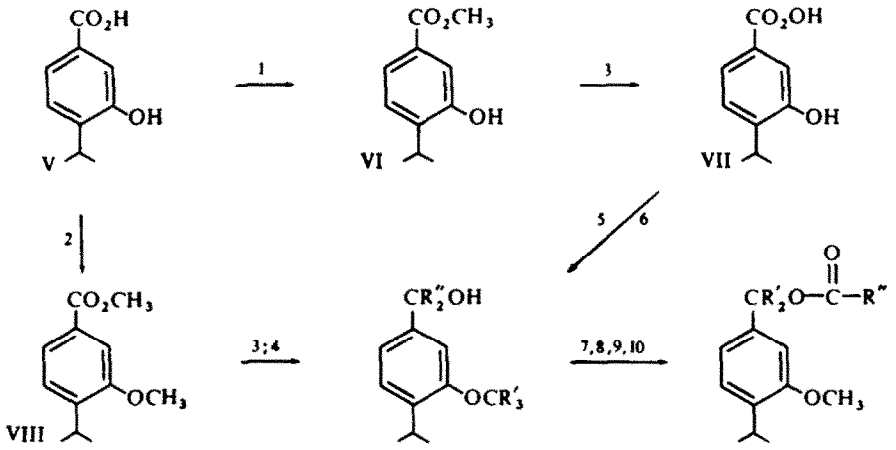
The antiseptic properties of the oxygenated thymol derivatives are currently being investigated.

EXPERIMENTAL

M.ps determined on a Thomas Hoover capillary m.p. apparatus and b.ps are uncorrected. IR spectra were recorded on a Perkin–Elmer Infracord model 337 spectrophotometer equipped with a NaCl prism. NMR spectra were taken on a Varian HA-100 spectrometer for 5–10% solns in $CDCl_3$ containing TMS as an internal standard. Mass spectra were taken with an Hitachi Perkin–Elmer RMU 6 instrument, the samples being introduced directly into the ion source through a vacuum-lock, electron energy 70 eV, electron current 20 μA, source temp < 120°, secondary electron multiplier as the detector. Refraction indices were determined on Carl Zeiss Refractometer 13234. Elemental analyses were performed by the microanalytical laboratory of the Weizmann Institute of Science.

Isolation procedure. Air dried young roots of *Inula viscosa* Ait (100 g) were extracted with successive batches of light petroleum (60°/80°) at room temp. Evaporation of the solvent yielded a coloured oily residue (2·5 g) which, prior to vacuum distillation, was rapidly chromatographed through a neutral alumina (grade II) column, using light petroleum as the eluent. Distillation of the chromatographed light petroleum extract (110–125°/0·2 mm) yielded an oily, yellow coloured distillate (*ca* 1 g), from which I, II and III were separated on a Perkin–Elmer Preparative Gas Chromatograph, Model F-21. The stationary phase consisted of SE-30 (5%) supported on Gaschrom-W 100/120 mesh, in a 6 ft. column kept at 150°. The retention times for I, II and III were: 18, 30 and 45 min respectively.

3-Hydroxycumic acid, methyl ester (VI). Compound V (10 g) dissolved in MeOH (50 ml) in the presence of a few drops of conc H_2SO_4 was refluxed for 6 hr. Following the usual work-up, VI was obtained in quantitative yield, b.p. 100°/0·04 mm, and crystallized upon cooling to room temp; $\nu_{max}^{CHCl_3}$ 1700, 1620,



IV R' = R'' = H (3,5) IR' = H R'' = $CHMe_2$

d_2-IV R' = H'' R'' = D (4) d_2-I R' = D R'' = $CHMe_2$

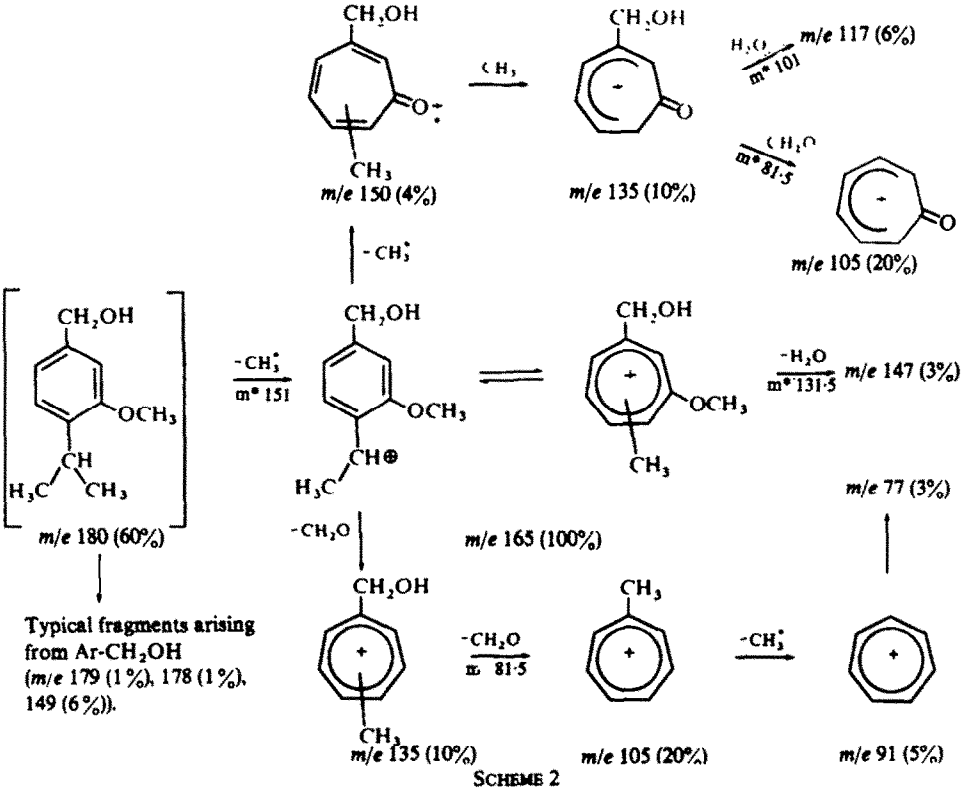
II R' = H R = CH_2CHMe_2

d_3-IV R' = D R'' = H (6) IX R' = H R'' = CH_3

d_3-IX R' = H R'' = CD_3

Reagents: 1. MeOH, H^+; 2. CH_2N_2 ether, 48 hr; 3. $LiAlH_4$; 4. $LiAlD_4$; 5. CH_3I, K_2CO_3; 6. CD_3I, K_2CO_3; 7. $(CH_3)_2CHCOCl$; 8. $(CH_3)_2CHCH_2COCl$; 9. CH_3COCl, ether, Pyr; 10. CD_3COCl, $ether_2$–Pyr.

SCHEME 1



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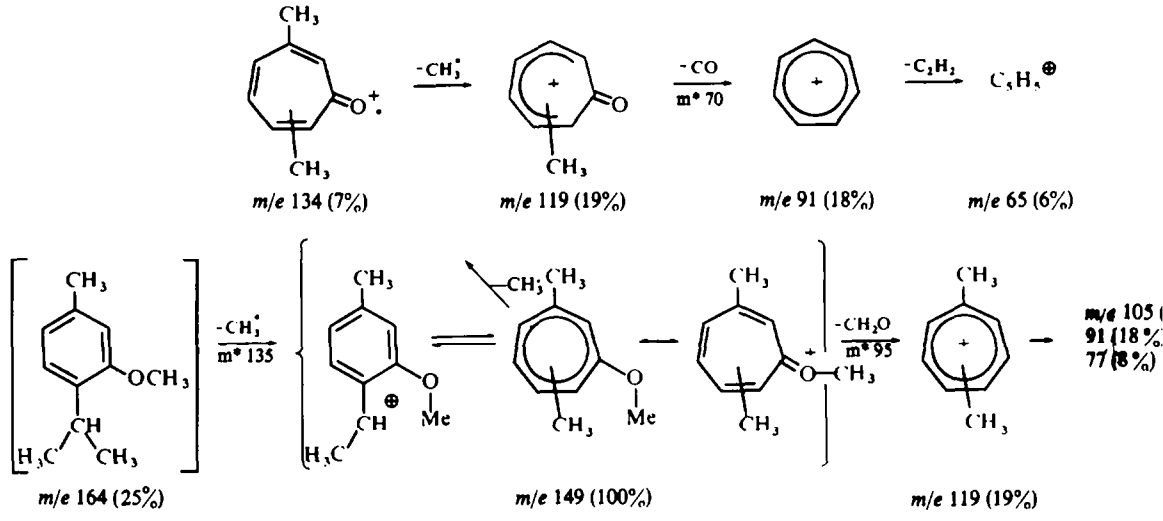

SCHEME 3

1580, 1430, 1280, 1170, 940, 880 cm^{-1}. (Found: C, 68·00; H, 7·30. $C_{11}H_{14}O_3$ requires: C, 68·02; H, 7·27%).

3-Methoxycumic acid, methyl ester (VIII). Treatment of V (10 g) with an excess of diazomethane in ether (prepared from 50 g nitrosomethylurea and added twice over a period of 48 hr at room temp), yielded upon the usual work-up VIII, as a colourless oil in quantitative yield; b.p. 95°/0·5 mm; ν_{max}^{neat} 1700, 1590, 1570, 1495, 1290, 1270, 1230, 1110, 1040, 990, 870, 760 cm^{-1}. (Found: C, 69·18; H, 7·59. $C_{12}H_{16}O_3$ requires: C, 69·21; H, 7·74%).

3-Hydroxy-4-isopropylbenzenylalcohol (p-*cymene*-3,7-*diol*) (VII). Reduction of VI (10 g) in dry ether (150 ml) with LAH (3 g), yielded upon the usual work-up VII as a colourless crystalline material in quantitative yield, m.p. 65–66° (benzene–light petroleum); $\nu_{max}^{CHCl_3}$ 3580, 3330, 2950, 2860, 1610, 1580, 1430, 1280, 1230, 1150, 1080, 1050, 1000, 930, 860, 810 cm^{-1}. Found: C, 75·25; H, 8·47. $C_{10}H_{14}O_2$ requires: C, 72·26; H, 8·49%).

3-Methoxy-4-isopropylbenzylalcohol (p-*cymene*-3-*methoxy*-7-*ol*) (IV). Reduction of VIII (10 g) with LAH (2 g) or treatment of VII (10 g) with an excess of MeI (12 g) in the presence of K_2CO_3 (12 g) in acetone (150 ml) yielded IV as a colourless oil, in quantitative yield, b.p. 94°/0·5 mm, $n_D^{18°}$ = 1·5260; $\nu_{max}^{CHCl_3}$ 3565, 3370, 2935, 1600, 1570, 1490, 1460, 1415, 1250, 1160, 1090, 1070, 1040, 850, 818 cm^{-1}. (Found: C, 73·30; H, 8·81. $C_{11}H_{16}O_2$ requires: C, 73·30; H, 8·95%).

Benzyl-deuterated 3-methoxy-4-isopropylbenzylalcohol (d_2-IV). Employing $LiAlD_4$ in the reduction of VIII as described above, gave d_2-IV; $\nu_{max}^{CCl_4}$ 3580, 3300, 2940, 2170, 2070, 1600, 1570, 1500, 1450, 1400, 1250, 1170, 1090, 1040, 1020 cm^{-1}. (Found: M^+ 182. $C_{11}H_{14}D_2O_2$ requires: M^+ 182).

Methoxy-deuterated 3-methoxy-4-isopropylbenzylalcohol (d_3-IV). Treatment of VII with deuterated MeI in acetone, in the presence of anhyd K_2CO_3 yielded d_3-IV; $\nu_{max}^{CCl_4}$ 3580, 3320, 2940, 2850, 2210, 2070, 1610, 1580, 1500, 1430, 1260, 1165, 1110, 1060, 1000, 850 cm^{-1}. (Found: M^+ 185. $C_{11}H_{13}D_3O_2$ requires: M^+ 185).

General preparation of the esters I, II, III *and* IX. To a cooled soln of IV (1 g), or nerol (1 g), dissolved in dry ether (10 ml) and containing a few drops of pyridine was added the required amount of acyl halide (1 g) (acetyl, isobutyryl- or isovalerylchloride). After 1 hr at room temp, the mixture was poured into ice-water, the product extracted with ether, washed successively with HCl (1:4), H_2O, $NaHCO_3$ (5%), H_2O, dried and evaporated. All the esters distilled at a bath temp of 110–120°/0·2 mm. Compound I: Found: C, 72·00; H, 8·90. $C_{15}H_{22}O_3$ requires: C, 71·97; H, 8·86%; Compound II: Found: C, 72·75; H, 9·20. $C_{16}H_{24}O_3$ requires: C, 72·69; H, 9·15%; Compound III: Found: C, 76·05; H, 11·05. $C_{15}H_{26}O_2$ requires: C, 75·58; H, 11·00%; Compound IX: Found: C, 70·30; H, 8·21. $C_{13}H_{18}O_3$ requires: C, 70·24; H, 8·16%.

Acetyl-deuterated 3-methoxy-4-isopropylbenzylacetate (d_3-IX). Crude deuterated acetyl chloride, prepared by treating deuterated AcOH (1·0 equiv) with $SOCl_2$ (0·8 equiv), was reacted with IV in dry ether

in the presence of pyridine. The deuterated acetate was isolated in the usual manner; $\nu_{max}^{CCl_4}$ 2900, 1735, 1600, 1570, 1490, 1460, 1410, 1250, 1165, 1075, 1045, 970, 910, 850 cm^{-1}. (Found: M^+ 225. $C_{13}H_{15}D_3O_3$ requires: M^+ 225).

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REFERENCES

[1] D. S. Noyce and L. J. Dolley, *J. Org. Chem.* **26**, 1732 (1961)
[2] F. Bohlman, U. Niedballa and J. Schulz, *Chem. Ber.* **102**, 864 (1969)
[3] H. Budzikiewicz, C. Djerassi and D. H. Williams, *Mass spectrometry of organic compounds* pp. 81, 237. Holden-Day (1967)
[4] E. von Sydow, *Acta Chem. Scand.* **17**, 2504 (1963)