Diels–Alder Reactions. Part III.[1] Condensation of Methyl *trans*-β-Formylcrotonate with Retinol Acetate, with a Note on the Structure and Stereochemistry of Kitol

By **Barend V. Burger** and **Christoph F. Garbers,*** Department of Chemistry, University of Stellenbosch, Republic of South Africa

The structure of kitol (5), a natural retinol dimer, has been determined. Kitol was converted into a perhydro-*cis*-γ-lactone (20), suggesting *cis*-orientation of its two hydroxymethyl groups. Three model compounds were obtained by the Diels–Alder reaction of retinol acetate with methyl *trans*-β-formylcrotonate (9).

SINCE the discovery of kitol,[2] a natural retinol dimer, in the nonsaponifiable material from whale liver oil, a large number of papers on its chemistry have been published.[3] Kitol yields retinol and pseudokitol on pyrolysis[4] and Japanese authors have suggested structures (3) and (4) for kitol[4,5] and pseudokitol[4] respectively. Structure (3) was ascribed to the photodimer of retinol.[6] Structure (5) has since been established for kitol[7,8] and the photodimerisation of retinol has also been investigated in greater detail.[9,10]

Kitol concentrates yield crystalline kitol[11] in low yield (<10%) and although the name kitol was initially proposed for the kitol concentrate,[12] this name was retained for the crystalline material isolated from the concentrate.[11,13] The identity of the remaining material is still unknown, and it was suggested[11] that this behaviour may indicate the presence of geometric isomers of kitol in the concentrate.

Since the u.v. spectrum of kitol is not changed by irradiation in the presence of catalytic amounts of iodine, the intense absorption in the 220—250 nm region seems to support the presence of the trienyl chromophore R. Summation of the u.v. spectra[14] of 3-methyl-5-(2,6,6-trimethylcyclohex-1-enyl)penta-*trans*-2,4-dienol (6) and ethyl 3-hydroxy-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-*trans*-4,*trans*-6,*trans*-8-trienoate (7) † gives a spectrum with $\lambda_{max.}$ 290 nm (ε 39,000), and an ε value of 19,000 at 240 nm. These values are in good agreement with those published for kitol, suggesting as structures (4) or (5); consequently its structure was reinvestigated.

The kitol used in the present studies was isolated by a procedure analogous to that of Clough *et al.*[11] using molecular distillation and chromatography, as well as by a method employing chromatographic procedures only. It was recrystallised from ethyl formate [m.p. 138—139°, $\lambda_{max.}$ (EtOH) 295 nm (ε 49,000)]. On pyrolysis it yielded retinol. The n.m.r. spectrum of kitol is compatible with structures (4) and (5), but not with structure (3). When the signals associated with the methyl groups of the β-ionone rings are assigned, a signal corresponding to one methyl group in a saturated environment remains (τ 8·87, *cf.* Table). Moreover the distinction evident in each case between the 9- and 9′-methyl groups (τ 8·23 and 8·13), the 7,8- and 7′,8′-olefinic protons (τ 4·06 and 4·02), and the hydroxymethylene groups (multiplet at τ *ca.* 6·28) suggested that the molecule was unsymmetrical.‡

In an attempt to determine the relative orientation of the two hydroxymethyl groups, the i.r. spectrum of kitol was investigated. Concentrated solutions in chloroform showed OH bands at $\nu_{max.}$ 3630, 3570, and *ca.* 3270 cm^{-1}. On dilution the intensity of the last decreases, whereas the position of the weaker bands at 3630 and 3570 cm^{-1} is unchanged. By correlation with the i.r. spectra of *cis*- and *trans*-1,2-bishydroxymethylcyclohexane (free OH at *ca.* 3630 cm^{-1}),[15] the bands at 3630 and 3570 cm^{-1} can be ascribed to the free and intramolecularly hydrogen-bonded hydroxy-groups. This indicated that in kitol the hydroxymethyl groups were either 1,2-*cis*, 1,2-*trans*, or 1,3-*cis*. Furthermore, kitol was hydrogenated to perhydrokitol and the latter oxidised with chromic acid[16] to yield a lactone, $\nu_{max.}$ 1753 cm^{-1}, and an acid, $\nu_{max.}$ 1706 cm^{-1}, of which the former was isolated. Since the lactone absorption lies between the regions quoted by Bellamy[17] for γ-

† U.v. spectral properties of alcohol (6): $\lambda_{max.}$ 240 and 265 nm (ε 12,800 and 13,200), and hydroxy-ester (7): $\lambda_{max.}$ 290 nm (ε 30,200).

‡ For convenience the numbering used corresponds to that shown in structure (5).

[1] Part II, B. V. Burger, C. F. Garbers, E. du Plessis, and K. G. R. Pachler, preceding paper.

[2] H. Pritchard, H. Wilkinson, J. R. Edisbury, and R. A. Morton, *Biochem. J.*, 1937, **31**, 258.

[3] For a summary of the earlier literature see R. Kaneko, *Rep. Govt. Chem. Ind. Res. Inst. Tokyo*, 1962, **57**, 194.

[4] Y. Omote, *J. Chem. Soc. Japan*, 1959, **80**, 191; *Bitamin*, 1963, **28**, 267.

[5] R. Kaneko, *J. Chem. Soc. Japan*, 1959, **80**, 177.

[6] R. Kaneko, *J. Chem. Soc. Japan*, 1958, **79**, 1459; 1960, **81**, 1876.

[7] Preliminary communication, B. V. Burger, C. F. Garbers, K. Pachler, R. Bonnett, and B. C. L. Weedon, *Chem. Comm.*, 1965, 588.

[8] C. Giannotti, B. C. Das, and E. Lederer, *Chem. Comm.*, 1966, 28; *Bull. Soc. chim. France*, 1966, 3299.

[9] M. Mousseron-Canet, J. C. Mani, C. Favie, and D. Lerner, *Compt. rend.*, 1966, **262***C*, 163; M. Mousseron-Canet, *Adv. Photochem.*, 1966, **4**, 217; M. Mousseron-Canet, J. C. Mani, and D. Lerner, *Bull. Soc. chim. France*, 1966, 3043.

[10] C. Giannotti, *Canad. J. Chem.*, 1968, **46**, 3025.

[11] F. B. Clough, H. M. Kascher, C. D. Robeson, and J. G. Baxter, *Science*, 1947, **105**, 436.

[12] N. D. Embree and E. M. Shantz, *J. Amer. Chem. Soc.*, 1943, **65**, 910.

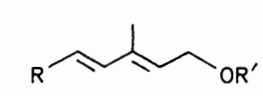
[13] H. Chatain and M. Debodard, *Compt. rend.*, 1951, **233**, 105; H. Chatain and M. A. Fridenson, *ibid.*, 1952, **234**, 1094.

[14] H. O. Huisman, A. Smit, P. H. van Leeuwen, and J. H. van Rij, *Rec. Trav. chim.*, 1956, **75**, 977.

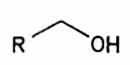
[15] L. P. Kuhn, *J. Amer. Chem. Soc.*, 1952, **74**, 2492.

[16] K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 1946, 39.

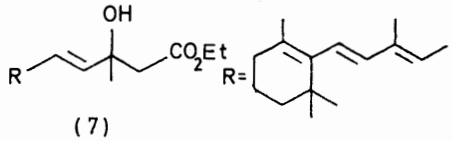
[17] L. J. Bellamy, ' The Infrared Spectra of Complex Molecules,' Methuen, London, 1958, p. 178.



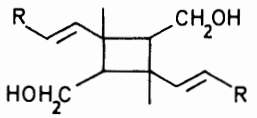
(1) R′= H
(2) R′= Ac



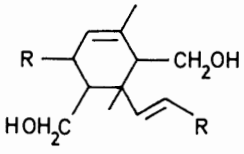
(6)



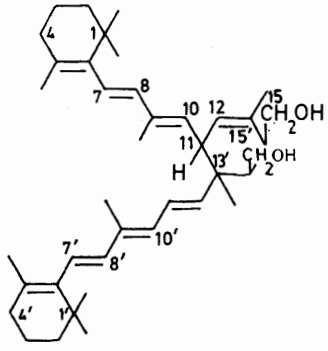
(7)



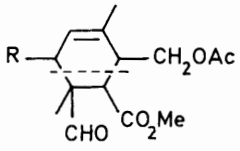
(3)



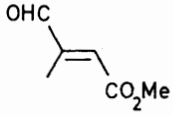
(4)



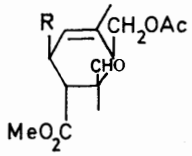
(5)



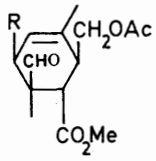
(8)



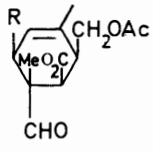
(9)



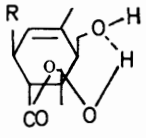
(10)



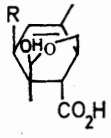
(11)



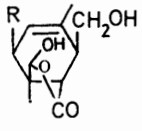
(12)



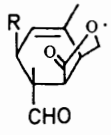
(13)



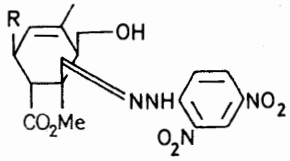
(14)



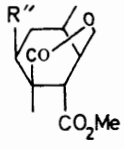
(14a)



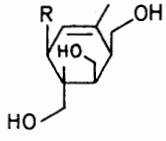
(15)



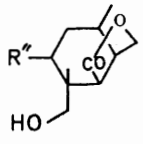
(16)



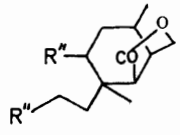
(17)



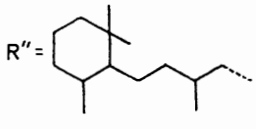
(18)



(19)



(20)



and δ-lactones (1780—1760 and 1750—1735 cm^{-1} respectively), the synthesis of model compounds and dimers of retinol was attempted.

It is known that retinol (1), retinol acetate (2), and retinol palmitate give 11,14-mono- and 7,10:11,14-di-adducts with maleic anhydride.[18] The monoadducts are formed much more readily, and consequently the synthesis of the monoadducts (8) through addition of retinol acetate (2) to methyl *trans*-β-formylcrotonate (9) was undertaken. A product was obtained in 28% yield which, according to its n.m.r. spectrum, contained at least two structurally different aldehydes (τ 0·41 and 0·47). In order to determine the structures of these adducts, the product was saponified. The hydrolysis products, lactol (13), hemiacetal (14), and γ-lactonealdehyde (15) were separated by chromatography on silica gel. These products showed u.v. absorption spectra typical of the triene system present in the 2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl)buta-1,3-dienyl group. The presence of this group was also clearly demonstrated by the respective n.m.r. spectra (see Table).

^1H N.m.r. (τ) data for kitol and model compounds

Compd.	Assignment of methyl resonances					
Kitol [a]	8·13 (C-9′)	8·22 (C-9 + C-13)	8·34 (C-5 + C-5′)	8·87 (C-13′)	8·99 (2 C-1 + 2 C-1′)	
(6) [b]	8·18 (C-9)		8·33 (C-5)		9·00 (2 C-1)	
(7) [b]	8·11 (C-9)		8·32 (C-5)		8·99 (2 C-1)	
(13) [c]	8·16 (C-9)	8·22 (C-13)	8·31 (C-5)	8·85 (C-13′)	8·97 (2 C-1)	
(15) [b]	8·17 (C-9)		8·30 (C-5 + C-13)	8·71 (C-13′)	8·98 (2 C-1)	
(18) [b]	8·17 (C-9)		8·32 (C-5 + C-13)		8·98 (2 C-1)	9·20 (C-13′)

[a] In CCl_4–$CDCl_3$ (3 : 1). [b] In $CDCl_3$. [c] In Me_2CO.

The lactol (13) shows a carbonyl absorption band at $\nu_{max.}$ 1770 cm^{-1}, which on hydrogenation shifts to $\nu_{max.}$ 1763 cm^{-1}. These frequencies appear to be rather low for a *trans*-γ-lactone, but introduction of a hydroxy-group in the γ-position of γ-lactones has been observed to cause shifts of 10 cm^{-1} to lower frequencies.[19] Stable five-, six-, and seven-membered lactol rings are known.[19,20] The lactol (13) gave a 2,4-dinitrophenylhydrazone (16), $\nu_{max.}$ 1700 cm^{-1}, which resisted lactonisation even after heating for 1 h at 100°. It would therefore appear that the hydroxymethyl and carboxy-groups are not in a favourable position for lactonisation. The above data could be interpreted in terms of the alternative structure (14a), which has the same spatial arrangement of functional groups as in product (14). Successive treatment of the lactol (13) with alkali and acid, however, led only to the recovery of this lactol, while no formation of the hemiacetal-acid (14) could be detected. The n.m.r. spectrum of the lactol (13) in acetone shows a one proton doublet at τ 3·59 (*J* 5 Hz) which disappeared on addition of deuterium oxide. Furthermore, the doublet at τ 4·72 (*J* 5 Hz), which is ascribed to a proton on a carbon atom attached to two oxygen atoms, merged to a singlet at τ 4·69 on addition of deuterium oxide. This can be explained by assuming the formation of a γ-lactol, the hydroxy-group of which is again intramolecularly hydrogen bonded to the primary hydroxy-group present. Coupling between a hydroxy-group and α-hydrogen atoms as a result of intramolecular hydrogen bonding, or the use of certain polar solvents is known.[21] The i.r. data for the lactol (13) is in agreement with this deduction. At high concentrations in chloroform the absorption bands ascribed to the OH stretching vibration appear as a sharp band at $\nu_{max.}$ 3600 cm^{-1} and a broad band at $\nu_{max.}$ *ca.* 3400 cm^{-1}. For dilute solutions the absorption band at $\nu_{max.}$ 3400 cm^{-1} disappears, and a concomitant shift in the CO stretching frequency from $\nu_{max.}$ 1767 cm^{-1} to 1773 cm^{-1} is observed. The absorption band at lower wavenumber can be ascribed to intermolecular hydrogen bonding (probably involving the carbonyl group), whereas the absorption band at $\nu_{max.}$ 3600 cm^{-1} is due to both the free primary hydroxy- and the intramolecularly bonded lactol hydroxy-groups. In CCl_4 the corresponding absorption bands for *cis*- and *trans*-1,2-bishydroxymethylcyclohexane occur at $\nu_{max.}$ 3633 and 3631 cm^{-1} respectively.

The acid (14), m.p. 172—174°, absorbed at $\nu_{max.}$ 1703 cm^{-1} and its n.m.r. spectrum (in acetone) did not show a resonance due to the presence of an aldehyde group; but the one proton resonance at τ 5·17 (*J* *ca.* 1 Hz) can be ascribed to a proton on a carbon atom attached to two oxygen atoms in a hemiacetal grouping. The product was hydrogenated, esterified with diazomethane, and oxidised with chromic acid to give the δ-lactone-ester (17) ($\nu_{max.}$ 1725 and 1732sh cm^{-1}; intensity enhanced), which provides additional evidence in favour of structure (14).

Finally, the resinous product (15), contained an aldehyde group, since it showed a single proton singlet in its n.m.r. spectrum at τ 0·61, and i.r. absorption bands at $\nu_{max.}$ 1721 and 2700 cm^{-1}. The absorption band at $\nu_{max.}$ 1762 cm^{-1} is ascribed to the presence of a *cis*-γ-lactone. In a qualitative experiment the product (15) was converted into a thioacetal and then heated with ethanolic ammonia, a procedure known to leave *cis*-γ-lactones unchanged, but which converts *trans*-γ-

[18] P. A. Plack, *Biochem. J.*, 1956, **64**, 56; K. Kawakami, *Sci. Papers Inst. Phys. Chem. Res., Tokyo*, 1935, **26**, 77 (*Chem. Abs.*, 1935, **29**, 2545); S. Hamano, *ibid.*, p. 87 (*Chem. Abs.*, 1935, **29**, 2545); C. D. Robeson and J. G. Baxter, *J. Amer. Chem. Soc.*, 1947, **69**, 136; C. D. Robeson ,J. D. Cawley, L. Weisler, M. H. Stern, C. C. Eddinger, and A. J. Chechak, *ibid.*, 1955, **77**, 4111; W. J. Serfontein, S. Brümmerhoff, and J. H. Jordaan, *J. S. African Chem. Inst.*, 1963, **16**, 22.

[19] *E.g.*, D. D. Wheeler, D. C. Young, and D. S. Erley, *J. Org. Chem.*, 1957, **22**, 547; V. W. Floutz, *ibid.*, 1960, **25**, 643.

[20] *E.g.*, B. E. Cross, R. H. B. Galt, and J. R. Hanson, *J. Chem. Soc.*, 1963, 5052; E. S. Hansen and H. H. Zeiss, *J. Amer. Chem. Soc.*, 1955, **77**, 1643.

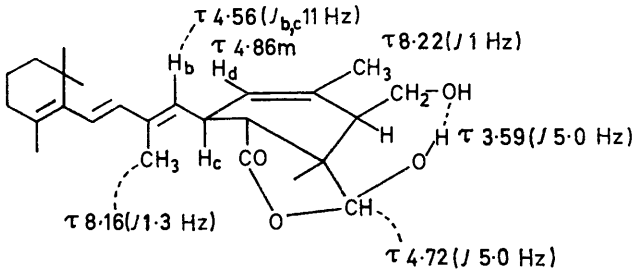
[21] *E.g.*, F. Hruska, T. Schaefer, and C. A. Reilly, *Canad. J. Chem.*, 1964, **42**, 697.

lactones into the corresponding hydroxy-amides.[22] This treatment had no effect on the thioacetal of lactone (15). On saponification of the lactone (15), followed by acidification with phosphoric or sulphuric acid, the lactone (15) was immediately reformed. Lithium aluminium hydride reduction gave the triol (18), and the hydroxy-*cis*-γ-lactone (19) ($\nu_{max.}$ 1755 cm^{-1}) was obtained by catalytic hydrogenation of lactone (15).

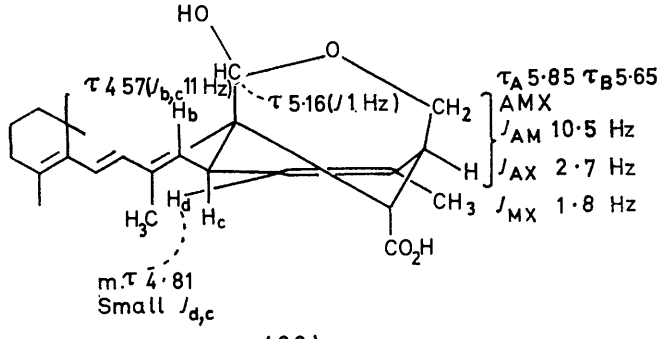
With acceptance of the validity of the *cis*-principle in the Diels–Alder reaction,[23] and on the assumption that no unexpected stereomutations took place during the synthesis, isolation, and structural elucidation of the adducts, the conformational formulae (21)—(23) can be proposed from the n.m.r. data for products (13)—(15) respectively. For both products (13) [*cf.* (21)] and (14) [*cf.* (22)] the small coupling between H_c and H_d shows that the trienyl group R is in a pseudo-equatorial position. However, with Dreiding models it can be demonstrated that for lactone (15) two conformational formulae, with the trienyl group in pseudo-axial or pseudo-equatorial position, should be considered. For the lactone (15) [*cf.* (23)] the coupling constant $J_{c,d}$ 10·3 Hz indicates a very small dihedral angle whereas the coupling constant $J_{b,c}$ 4 Hz suggests a dihedral angle between H_b and H_c approaching 90° as required by the Karplus equations.[24] It would therefore appear that, due to steric relationships the trienyl group is in pseudo-axial position with the plane of the triene-system approximately parallel to that of the lactone ring. In the triol (18) only a small coupling between protons H_c and H_d is observed, indicating a conformational change, with the group R now in pseudo-equatorial position. The lactone ring in product (15) probably stabilises the trienyl group R in the sterically less favourable pseudo-axial position.

The identity of the hydrolysis products (13)—(15) indicates the formation of adducts (10)—(12) in the Diels–Alder reaction between retinol acetate and methyl *trans*-β-formylcrotonate (9). These products were formed in the approximate ratio of 23 : 20 : 7 respectively. Mixtures of adducts were to be expected in the reaction between the trisubstituted dienophile (9) and the 1,2,4-trisubstituted ' diene ' (2).[23]

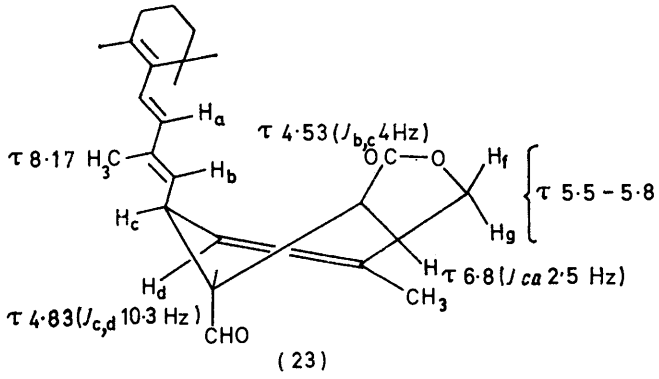
On the basis of the excellent agreement between the wavenumbers of the carbonyl stretching vibration of the γ-lactone (20) ($\nu_{max.}$ 1753 cm^{-1}), obtained by oxidation of perhydrokitol, and of the γ-lactone (19) ($\nu_{max.}$ 1755 cm^{-1}), synthesised by perhydrogenation of the aldehyde-lactone (15), as well as the position of the CO stretching vibrations of the δ-lactone-ester (17) ($\nu_{max.}$ 1725 and 1732sh cm^{-1}), structure (5) with a *cis*-orientation of the two hydroxymethyl groups was suggested for kitol.[2] This structure was confirmed by evidence based principally on mass spectrometry.[8] Furthermore, the photodimerisation of retinol acetate was studied and it was found that apart from cyclobutane derivatives of type (3), structures of type (5) are also formed.[9,10] Regarding the relative orientation of substituents on the central cyclohexene ring of kitol, attention should be drawn to the remarkable similarity between the two single proton doublets (τ 4·58, *J* 6 Hz and τ 4·86, *J* 10·5 Hz), attributed to protons at C-10 and C-12 in the



(21)



(22)



(23)

n.m.r. spectrum of kitol, and those of H_b and H_d (τ 4·53, *J* 4 Hz and τ 4·83, *J* 10·3 Hz) in the spectrum of lactone (15). This suggests a similar stereochemistry for the group R in both these compounds, *i.e.* in the pseudo-axial conformation. Despite many efforts we have been unable to convert the lactone (15) into a retinol dimer. In conclusion it should be mentioned that kitol has four asymmetric carbon atoms and thus would be expected to be optically active: no optical rotation of a 0·5% solution of kitol in methanol could be detected at wavelengths greater than 320 nm.

[22] M. S. Newman and C. A. Vander Werf, *J. Amer. Chem. Soc.*, 1945, **67**, 233.
[23] J. Sauer, *Angew. Chem. Internat. Edn.*, 1967, **6**, 16.
[24] L. M. Jackman and S. Sternhell, ' Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' Pergamon, Oxford, 2nd edn., 1969, p. 280.

$[\alpha]_D$ Values of −1·35 to −2·6° for kitol and −4° for its diacetate have been reported for more concentrated solutions.[8]

EXPERIMENTAL

Combustion analyses were by Dr. F. Pascher, Bonn. I.r. spectra were recorded on a Perkin-Elmer model 21 spectrometer using chloroform as solvent. U.v. spectra were recorded for solutions in 96% ethanol. ^{1}H N.m.r. spectra were recorded with a Varian A-60 spectrometer with tetramethylsilane as internal reference. M.p.s were determined on a Fischer m.p. apparatus.

Diels–Alder Addition of Methyl trans-β-*Formylcrotonate* (9) *to Retinol Acetate* (2).—A mixture of methyl *trans*-β-formylcrotonate (9) (31·2 g, 0·244 mol), retinol acetate (2) (20·6 g, 0·063 mol), and a drop of α-tocopherol was heated at 90° for 16 h. The decrease in concentration of reactants (2) and (9) was accompanied by formation of byproducts, and on termination of the reaction the crude mixture showed maxima at 368, 340, 325, 305, and 275 nm, as well as a strong maximum at 228 nm (β-formylcrotonate). Excess of β-formylcrotonate was removed under reduced pressure (40° and 0·15 mmHg), the residue (28·4 g) dissolved in iso-octane (22 ml), and mixed with non-wetting Kieselguhr (50 g). This was placed on a column packed in 50% methanol–water (v/v; saturated with iso-octane) with non-wetting Kieselguhr (204 g) impregnated with iso-octane (184 ml) at 15°. The column was eluted successively with 70 (5 l), 80 (7·25 l) and 90% (0·5 l) methanol–water (saturated with iso-octane). Fractions showing u.v. absorption in the 250—280 nm region were partly eluted by the 80% but mostly by the 90% MeOH–H_2O eluant. These fractions were combined, diluted with water, and extracted with light petroleum. The combined extracts were dried and the solvent evaporated to yield a residue (14·26 g) which was further purified by reversed-phase partition chromatography in a similar way. Elution was effected with 80% MeOH–H_2O (9·5 l). Fractions showing $\lambda_{max.}$ *ca.* 265 nm were combined and the adducts were isolated as before to yield a viscous product (7·99 g, 28%). A sample was purified by chromatography on alumina and the solvent was removed at 30—40° and 10^{-5} mmHg (6 h) to yield a mixture of the pure adducts, $\lambda_{max.}$ 266 nm (ε 17,100), $\nu_{max.}$ ($CHCl_3$) 1725 cm^{-1} (Found: C, 73·3; H, 8·75. Calc. for $C_{28}H_{40}O_5$: C, 73·6; H, 8·8%).

Saponification of the Diels–Alder Adducts.—A solution of potassium hydroxide (400 mg) in water (0·4 ml) was added to the mixture of adducts (1 g) in MeOH (10 ml). Subsequently water (*ca.* 10 ml) was added until the mixture became turbid. It was left for 52 h at room temperature, diluted with water, and non-saponifiable matter was extracted with ether. The aqueous phase was acidified with phosphoric acid, saturated with ammonium chloride, and extracted with ether. The combined extracts were washed, dried, and the solvent was evaporated to yield a product (862 mg), $\nu_{max.}$ ($CHCl_3$) 1763 and 1707 cm^{-1}, which was then adsorbed on silica gel (40 g) and eluted with $CHCl_3$ (450 ml), 10% EtOAc–$CHCl_3$ (150 ml), 20% EtOAc–$CHCl_3$ (150 ml), and EtOAc (300 ml). Fractions were analysed by i.r., and the following hydrolysis products were isolated and their structures established.

(a) cis-γ-*Lactone-aldehyde* (15). This product (114 mg), eluted with $CHCl_3$ and partly with EtOAc–$CHCl_3$ (1 : 9), was finally purified by chromatography on alumina (activity II), $\lambda_{max.}$ 260 nm (ε 16,200) and *ca.* 250sh nm (15,500), $\nu_{max.}$ ($CHCl_3$) 2700 (CHO), 1762 (cis-γ-lactone), and 1721 cm^{-1} (CHO) (Found: C, 78·5; H, 9·1. $C_{25}H_{34}O_3$ requires C, 78·5; H, 9·0%).

(i) Ammonolysis of the *cis*-γ-lactone thioacetal. The thioacetal of the γ-lactone-aldehyde (15) was prepared by adding anhydrous sodium sulphate (100 mg) and freshly fused zinc chloride (10 mg) to a cold (5°) solution of the γ-lactone-aldehyde (15) (10 mg) in ethyl mercaptan (0·2 ml). After keeping the mixture at 5° for 20 h and at room temperature for 4 h, it was diluted with ether and washed with water. Evaporation of the solvent gave the thioacetal which showed $\nu_{max.}$ ($CHCl_3$) 1761 cm^{-1} (γ-lactone) and no aldehyde bands. This thioacetal was dissolved in ethanol saturated with ammonia, the solution was sealed in a glass tube, and heated to 90° for 10 h. The ethanol and ammonia were removed under reduced pressure, and the recovered thioacetal, $\lambda_{max.}$ 255 nm (ε 15,500), showed an i.r. spectrum identical to the starting thioacetal.

(ii) Hydrogenation of the *cis*-γ-lactone-aldehyde (15). The aldehyde (15) (46 mg) in HOAc (10 ml) was hydrogenated over Adams catalyst. The hydrogen absorbed corresponded to 4·84 double bonds. The hydrogenated product was isolated in the usual way, adsorbed on silica gel (2 g), and the saturated cis-γ-*lactone-alcohol* (19) was eluted with ether–light petroleum (1 : 3). After drying for 1 h at 100° and 10^{-5} mmHg, the product showed $\nu_{max.}$ ($CHCl_3$) 3620 (free OH), 3465 (bonded OH), and 1755 cm^{-1} (cis-γ-lactone) (Found: C, 76·4; H, 11·3. $C_{25}H_{44}O_3$ requires C, 76·5; H, 11·3%).

(b) *Hemiacetal-acid* (14). The acid (323 mg), eluted with EtOAc–$CHCl_3$ (1 : 4), was recrystallised from $CHCl_3$ and from ether, m.p. 172—174°, $\lambda_{max.}$ 259 (ε 20,200) and *ca.* 250sh nm (19,800), $\nu_{max.}$ (saturated solution in $CHCl_3$) 3602 (free OH), 3520—3000 (bonded OH), and 1703 cm^{-1} (acid) (Found: C, 75·2; H, 9·0. $C_{25}H_{36}O_4$ requires C, 75·0; H, 9·1%).

(i) Conversion of hemiacetal-acid (14) into δ-lactone-ester (17). The hemiacetal-acid (14) (80·4 mg) in MeOH (10 ml) was hydrogenated with Adams catalyst (30 mg), hydrogen equivalent to 4 double bonds being taken up, and the product, $\nu_{max.}$ ($CHCl_3$) 1700 cm^{-1} (acid), was isolated. This was dissolved in ether (1 ml), the solution cooled to 0°, and treated with a slight excess of ethereal diazomethane. Evaporation of the solvent gave a saturated hemiacetal-ester, $\nu_{max.}$ ($CHCl_3$) 1723 cm^{-1} (ester), which was dissolved in acetone (1 ml) and to which chromic acid [0·06 ml of a solution of CrO_3 (39·887 g)–6N-H_2SO_4 (200 ml)] was then added at room temperature with stirring. After 55 min the mixture was diluted with water, saturated with ammonium chloride, and extracted with ether. From the extracts the crude δ-lactone-ester was isolated and purified by adsorption on alumina (activity I) and elution with ether (yield 60 mg), $\nu_{max.}$ ($CHCl_3$) 1725 and 1732sh cm^{-1} (ester and δ-lactone) (Found: C, 74·4; H, 10·05. $C_{26}H_{44}O_4$ requires C, 74·6; H, 10·3%).

(c) *Lactol* (13). The lactol (376 mg) was eluted with EtOAc, m.p. (needles from $CHCl_3$) 170°, $\lambda_{max.}$ 240 and 262 nm (ε 16,700 and 17,600), $\nu_{max.}$ (saturated solution in $CHCl_3$) 3600 (free OH), 3390 (bonded OH), and 1767 cm^{-1} (γ-lactone) (Found: C, 75·1; H, 9·05. $C_{25}H_{36}O_4$ requires C, 75·0; H, 9·1%).

(i) 2,4-Dinitrophenylhydrazone of lactol (13). To a stirred solution of lactol (13) (25 mg) dissolved in a minimum of EtOH, a solution (0·2 ml) of 2,4-dinitrophenyl-

hydrazine in phosphoric acid and EtOH was added. The crystals were filtered off, washed and dried, and recrystallised from ether–light petroleum to yield the 2,4-*dinitrophenylhydrazone*, m.p. 174—176° (sintering at 172·5°), $\lambda_{max.}$ 252 and 366 nm (ε 25,700 and 20,700), $\nu_{max.}$ ($CHCl_3$) 3300, 1700 (acid), 1618, 1591, 1332, 1310, 975, 917, and 832 cm^{-1} (Found: C, 64·2; H, 7·2. $C_{31}H_{40}N_4O_7$ requires C, 64·1; H, 6·9%). Heating the 2,4-dinitrophenylhydrazone at 100° for 1 h caused no change in the i.r. spectrum.

(ii) Hydrogenation of lactol (13). The lactol was hydrogenated in HOAc with Adams catalyst and purified by chromatography on silica gel. The saturated *lactol* showed $\nu_{max.}$ ($CHCl_3$) 1763 cm^{-1} (Found: C, 73·7; H, 11·0. $C_{25}H_{44}O_4$ requires C, 73·5; H, 10·8%).

(iii) Attempted conversion of lactol (13) into hemiacetal-acid (14). The lactol (90 mg) in EtOH (1 ml) was mixed with a solution of KOH (40 mg) in water (1 ml) and the mixture was left for 48 h. The solution was diluted with water and extracted with ether, the aqueous phase was acidified with 50% phosphoric acid, and again extracted with ether. The crystalline saponifiable material (67 mg) was identical (i.r.) with the starting lactol. The identity was further confirmed by m.p. and mixed m.p. after one recrystallisation from $CHCl_3$.

Isolation of Kitol.—(a) An extract (3 kg) of whale liver oil in peanut oil [25] was saponified according to the method of Boldingh and Drost [26] and the nonsaponifiable matter (96 g) was isolated. Steroids were largely removed by crystallisation from acetone at −20°. The noncrystalline material (52 g) was dissolved in peanut oil residue (780 g) and the retinol was separated by distillation at 150—160° in a cyclic molecular still.[27] The residue was saponified and the nonsaponifiable fraction [$E^{1\%}_{1\,cm}$ (293 nm) 343] was isolated and purified by crystallisation of steroids at low temperature. The residue (15 g) was chromatographed on alumina (600 g; activity III) to yield a kitol concentrate (7·6 g) [$E^{1\%}_{1\,cm}$ (293 nm) 646], which was further purified by successive reversed-phase partition chromatography and chromatography on deactivated alumina. The kitol fractions were dissolved in ethyl formate and cooled to −20°. Crystalline kitol was obtained in poor yield and recrystallised from ethyl formate, m.p. 138—139°, $\lambda_{max.}$ 296 nm (ε 50,600) (Found: C, 83·3; H, 10·2. Calc. for $C_{40}H_{60}O_2$: C, 83·85; H, 10·6%). Treatment of the noncrystalline material from the mother liquor [$E^{1\%}_{1\,cm}$ (294 nm) 677] with iodine did not improve crystallisation.

(b) Similarly, the nonsaponifiable material (188 g) from a peanut oil extract of whale liver oil (4·5 kg) was freed from sterols to yield a residue (116 g) which was chromatographed on alumina (3 kg; activity II) to give a kitol concentrate (29 g) [$E^{1\%}_{1\,cm}$ (300 nm) 627]. This was further purified by reversed-phase partition chromatography (stationary phase: iso-octane; mobile phase: aqueous MeOH), followed by column chromatography on alumina. The fractions containing kitol were crystallised as in (a) and gave crystalline kitol in poor yield, m.p. 140·5—141°, $\lambda_{max.}$ 296·5 nm (ε 51,400).

Pyrolysis of Kitol.—The crystalline kitol (1·9 mg) was heated to 220° in a glass tube at 5×10^{-3} mmHg for 3 min. The retinol formed was purified by reversed-phase partition chromatography, and showed $\lambda_{max.}$ (EtOH) 328 nm. This solution was made 0·033N with respect to hydrochloric acid by addition of 0·8N ethanolic hydrochloric acid and left at room temperature for 15 min, after which it was neutralised with ethanolic sodium hydroxide. The typical anhydrovitamin A spectrum ($\lambda_{max.}$ 349, 369, and 390 nm) was obtained.[28]

Oxidation of Perhydrokitol.—Crystalline kitol (16 mg) in absolute EtOH (5 ml) in the presemce of 5% Pd–C catalyst absorbed 8·4 double-bond equivalents of hydrogen to yield perhydrokitol. A solution of perhydrokitol (137 mg) in acetone (2 ml) was stirred and treated with a chromic acid solution (0·31 ml; *cf.* oxidation of hemiacetal-acid above). On completion of reaction, the mixture was diluted with water and the oxidation product was extracted with ether. The combined extracts were washed, dried, and the ether was evaporated. The crude oxidation product (100 mg) was chromatographed on silica gel (7 g) and the fractions analysed by i.r. The γ-lactone (20), which was eluted with ether–light petroleum (1 : 19), was not quite pure and was consequently rechromatographed on activated alumina. The pure *cis-γ-lactone* (20) (60 mg) was eluted with ether–light petroleum (1 : 4) and showed $\nu_{max.}$ ($CHCl_3$) 1753 cm^{-1} (Found: C, 81·8; H, 12·0. $C_{40}H_{72}O_2$ requires: C, 82·1; H, 12·4%). During the oxidation an acidic component ($\nu_{max.}$ 1705 cm^{-1}) was also formed as a minor product.

Financial assistance from the South African Council for Scientific and Industrial Research is gratefully acknowledged. We also thank South African Marine Products for generous gifts of whale liver oil extracts, Professor B. C. L. Weedon and Dr. R. Bonnett for their collaboration regarding the i.r. and n.m.r. studies on kitol, Professor W. Klyne for measuring the optical rotation, and Dr. K. G. R. Pachler for recording some of the n.m.r. spectra.

[2/2102 *Received*, 6*th September*, 1972]

[25] L. C. Surmon and M. F. Ovenden, *S. African Ind. Chemist*, 1962, **16**, 62.
[26] J. Boldingh and J. R. Drost, *J. Amer. Oil Chem. Soc.*, 1951, **28**, 480.
[27] K. C. D. Hickman, *Ind. and Eng. Chem.*, 1937, **29**, 968.
[28] E. M. Schantz, J. D. Cawley, and N. D. Embree, *J. Amer. Chem. Soc.*, 1943, **65**, 901; S. R. Ames and P. L. Harris, *Science*, 1954, **120**, 391.