Preparation and Properties of 5,6-Monoepoxyvitamin A Acetate, 5,6-Monoepoxyvitamin A Alcohol, 5,6-Monoepoxyvitamin A Aldehyde and their Corresponding 5,8-Monoepoxy (Furanoid) Compounds

BY F. B. JUNGALWALA AND H. R. CAMA
Department of Biochemistry, Indian Institute of Science, Bangalore 12, *India*

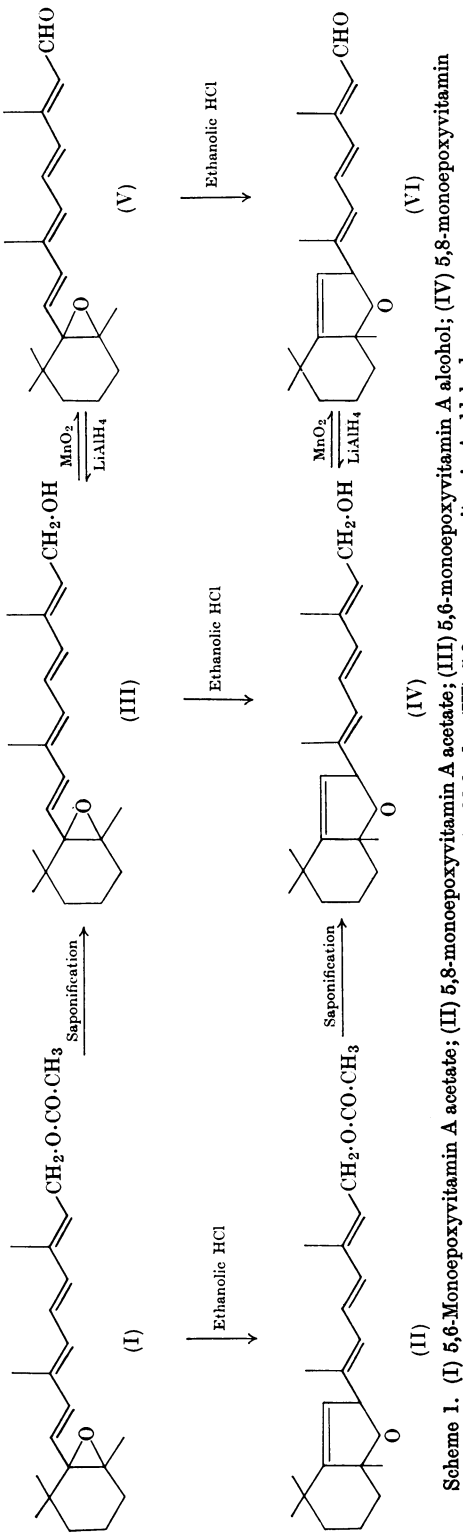
(*Received* 19 *May* 1964)

1. Oxidation of vitamin A acetate with monoperphthalic acid gave 5,6-monoepoxyvitamin A acetate, $C_{22}H_{32}O_3$, obtained as pale-yellow crystals, m.p. 65–66°. 2. Saponification of 5,6-monoepoxyvitamin A acetate yielded 5,6-monoepoxyvitamin A alcohol, which was readily oxidized with manganese dioxide to 5,6-monoepoxyvitamin A aldehyde, obtained as yellow crystals, m.p. 101–102°. It was the most stable of all the epoxy compounds studied. 3. Treatment of the 5,6-epoxy compounds with ethanolic hydrochloric acid gave the corresponding 5,8-epoxy (furanoid) compounds. 5,8-Monoepoxyvitamin A aldehyde was obtained as crystals, m.p. 104–105°, but was very unstable. 4. Crystalline semicarbazones and phenylhydrazones with constant melting points and characteristic spectra were prepared from 5,6- and 5,8-monoepoxyvitamin A aldehyde. 5. Reduction of 5,6- and 5,8-monoepoxyvitamin A aldehyde with lithium aluminium hydride gave the corresponding 5,6- and 5,8-monoepoxyvitamin A alcohol. 6. 5,6- and 5,8-Monoepoxyvitamin A aldehyde were fed to vitamin A-deficient rats, and the compounds obtained from the livers of rats were indistinguishable from the reduction products obtained with lithium aluminium hydride. 7. The structures of the epoxy compounds were confirmed by their chromatographic behaviour, elemental analyses, ultraviolet-, visible- and infrared-absorption spectra and nuclear-magnetic-resonance spectra.

A number of reports have described the presence of several substances which have absorption bands lower in the ultraviolet region ($\lambda_{max.}$ 275, 285, 297 and 310mμ) than that of vitamin A ($\lambda_{max.}$ 328mμ) and which give different coloured products with the antimony trichloride colour test. Such substances have been found in oxidized fish-liver oils (Euler, Karrer & Zubrys, 1934; Embree & Shantz, 1943; Halpern, 1946) and in autoxidized samples of pure vitamin A (Bolomey, 1947; Milas, 1947; Groot, 1949; Bagnall & Stock, 1952), as well as in unsaponifiable material of blood plasma, different organs and faecal material of animals and man after large oral doses of vitamin A (LePage & Pett, 1941). Pritchard, Wilkinson, Edisbury & Morton (1937) isolated a substance having a characteristic $\lambda_{max.}$ 285–295mμ from mammalian-liver-oil concentrates. The antimony trichloride reaction product had $\lambda_{max.}$ 594 and 496mμ.

Morton & Goodwin (1944) oxidized vitamin A alcohol with potassium permanganate and dilute sulphuric acid, and obtained vitamin A aldehyde (retinene) and a substance having $\lambda_{max.}$ 342mμ in light petroleum, and giving a colour with $\lambda_{max.}$ 560mμ on reaction with antimony trichloride. By employing the same treatment, a similar product was also obtained by Meunier & Jouanneteau (1948). Karrer & Jucker (1945, 1947) obtained 'hepaxanthin' by the action of monoperphthalic acid on vitamin A alcohol. The synthetic 'hepaxanthin' had $\lambda_{max.}$ 275mμ, and with antimony trichloride reagent it gave a pink product having $\lambda_{max.}$ 575mμ that immediately gave rise to a blue solution with $\lambda_{max.}$ 620mμ. They suggested that 'hepaxanthin' may be vitamin A epoxide. The other product of reaction of vitamin A and monoperphthalic acid was 'compound Y' having $\lambda_{max.}$ 339mμ, and with antimony trichloride it gave a pink product with $\lambda_{max.}$ 575mμ. In all the above studies, the true chemical nature and properties of the alleged vitamin A epoxide was not defined.

The present paper describes the preparation and properties of 5,6-monoepoxyvitamin A acetate, 5,6-monoepoxyvitamin A alcohol, 5,6-monoepoxyvitamin A aldehyde and their corresponding 5,8-monoepoxy (furanoid) compounds (Scheme 1).



Scheme 1. (I) 5,6-Monoepoxyvitamin A acetate; (II) 5,8-monoepoxyvitamin A acetate; (III) 5,6-monoepoxyvitamin A alcohol; (IV) 5,8-monoepoxyvitamin A alcohol; (V) 5,6-monoepoxyvitamin A aldehyde; (VI) 5,8-monoepoxyvitamin A aldehyde.

EXPERIMENTAL

Materials. Crystalline vitamin A acetate and alcohol (Roche Products Ltd., Welwyn Garden City, Herts.) were used. Light petroleum (b.p. 40–60°) was left over $KMnO_4$, washed, distilled, dried over $CaCl_2$ and redistilled before use. Diethyl ether was freshly distilled over reduced iron to remove any peroxides. Ethanol, for spectroscopic use, was refluxed with zinc dust and KOH and distilled. Cyclohexane, chloroform, acetone and carbon tetrachloride were spectroscopically pure solvents.

Alumina (E. Merck, Darmstadt, Germany) was deactivated with water (5–10%, v/w) by stirring slowly under light petroleum. Neutral alumina was prepared by soaking 500g. of alumina in 1l. of 0·1N-HCl and stirring for 30min., filtering and washing to neutral pH, and drying at 100° for 24hr. Monoperphthalic acid was prepared from sublimed phthalic anhydride and hydrogen peroxide (E. Merck) according to the method of Böhme (1955) and Royals & Harrell (1955).

Freshly prepared saturated solution (25%, w/v) of $SbCl_3$ was used for the Carr–Price reaction after the addition of 1% (v/v) of acetic anhydride just before use.

Spectroscopic measurements. The ultraviolet- and visible-absorption spectra of compounds were measured with a Beckman model DU spectrophotometer. Infrared-absorption spectra of substances dissolved in carbon tetrachloride [1% (w/v) solutions] were determined in 0·1cm. light-path NaCl cells with an Infracord (Perkin–Elmer) double-beam spectrometer. Nuclear-magnetic-resonance spectra were recorded on a Varian A-60 spectrometer, with tetramethylsilane as the reference standard, at the National Chemical Laboratory, Poona, India.

Oxidation of vitamin A acetate with monoperphthalic acid and isolation of 5,6-monoepoxyvitamin A acetate (I). Vitamin A acetate (4·0g.) was dissolved in dry diethyl ether, and 2·22g. (1:1 molar ratio) of freshly prepared monoperphthalic acid was added at room temperature in an atmosphere of N_2. The reaction was followed by examining the ultraviolet-absorption spectrum of the reaction mixture. After about 60hr. most of the vitamin A acetate had reacted and the ultraviolet-absorption spectrum of the reaction mixture showed $\lambda_{max.}$ 310mμ with a subsidiary peak at 325mμ. The reaction was arrested by the addition of dilute $NaHCO_3$ solution, and the ether layer was washed with distilled water, dried over anhydrous Na_2SO_4, filtered and reduced in volume under reduced pressure. The residue was dissolved in 50ml. of light petroleum and chromatographed on a 5%-(v/w)-water-deactivated alumina (200g.) column (3·5cm.×35cm.). The main fraction (Table 1: fraction 2) was rechromatographed on a 5%-(v/w)-water-deactivated alumina (100g.) column (3cm.×25cm.), when one homogeneous band was obtained. It was eluted with 5–7% (v/v) diethyl ether in light petroleum and had $E^{1\%}_{1cm.}$ 1500 at 310mμ. The compound crystallized at low temperature (−30°) from light petroleum. The m.p. of the pale-yellow crystals remained constant at 65–66° after four crystallizations from light petroleum (b.p. 40–60°) (Found: C, 76·38; H, 9·04; O, 14·58. $C_{22}H_{32}O_3$ requires: C, 76·18; H, 9·37; O, 14·45%).

Preparation of 5,8-monoepoxyvitamin A acetate (II). Crystalline 5,6-monoepoxyvitamin A acetate (500mg.) was dissolved in 50ml. of ethanol, and 3ml. of ethanolic 0·05N-HCl was added. After 3–5min. the isomerization had

taken place, as observed by the hypsochromic wavelength shift (30mμ) in the ultraviolet-absorption spectrum of the compound (Table 2). The reaction was stopped by the addition of dilute $NaHCO_3$ solution, and the products were extracted with diethyl ether. The ether extract was washed free of $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered and reduced in volume under reduced pressure. The concentrate was chromatographed on a 5%-(v/w)-water-deactivated alumina (100g.) column (3cm.×25cm.), when the 5,8-monoepoxyvitamin A acetate was eluted with 10–12% (v/v) diethyl ether in light petroleum. It was rechromatographed on 5%-(v/w)-water-deactivated alumina, when a single band having $\lambda_{max.}$ 280mμ ($E^{1\%}_{1cm.}$ 1000) in light petroleum was eluted. The yield was 80%. The substance was unstable and could not be kept at room temperature for long periods. It formed whitish gummy-like material at −30° when crystallization was attempted. Elementary analysis of the compound could not be performed owing to the instability of the compound.

Preparation of 5,6-monoepoxyvitamin A alcohol (III). 5,6-Monoepoxyvitamin A acetate (500mg.) was saponified with 6·0ml. of ethanolic 10% (w/v) KOH for 1hr. under an atmosphere of N_2. The product was extracted with diethyl ether, washed free of alkali, dried and the volume reduced *in vacuo*. The unsaponifiable fraction was unstable and was quickly chromatographed on a 7%-(v/w)-water-deactivated alumina (100g.) column (3cm.×25cm.). The 5,6-monoepoxyvitamin A alcohol formed from the 5,6-monoepoxyvitamin A acetate was eluted with 20–25% (v/v) diethyl ether in light petroleum (yield 350mg.). The unstable compound could not be crystallized or analysed.

Preparation of 5,8-monoepoxyvitamin A alcohol (IV). 5,6-Monoepoxyvitamin A alcohol (300mg.) was isomerized with ethanolic HCl to 5,8-monoepoxyvitamin A alcohol, as described for the preparation of 5,8-monoepoxyvitamin A acetate (yield, 210mg.). 5,8-Monoepoxyvitamin A alcohol was also obtained by saponification of 5,8-monoepoxyvitamin A acetate in 82% yield; it was purified by chromatography (twice) on a 7% (v/w)-water-deactivated alumina column, giving one band with $\lambda_{max.}$ 280mμ in light petroleum, with 25–30% (v/v) ether in light petroleum. The ultraviolet-absorption spectra of the chromatographically homogeneous substance in different solvents were taken immediately.

Preparation of 5,6-monoepoxyvitamin A aldehyde (V). 5,6-Monoepoxyvitamin A alcohol (500mg.) was oxidized with 3·0g. of MnO_2, according to the method of Ball, Goodwin & Morton (1948), and the reaction mixture was kept for 12hr. in the dark, when the $\lambda_{max.}$ changed from 310 and 325mμ to 352mμ; 450mg. of crude product ($\lambda_{max.}$ 352mμ in light petroleum) was obtained. The product was chromatographed on a 5%-(v/w)-water-deactivated alumina (100g.) column (3cm.×25cm.). The main band was eluted with 10–12% (v/v) diethyl ether in light petroleum. It was rechromatographed on 5%-(v/w)-water-deactivated alumina and the concentrate was crystallized from light petroleum (b.p. 40–60°). The m.p. of the yellow crystals remained constant at 101–102° after four crystallizations; the compound was stable, and detailed studies were carried out (Found: C, 79·63; H, 9·49; O, 10·88. $C_{20}H_{28}O_2$ requires: C, 79·95; H, 9·39; O, 10·66%).

Preparation of 5,6-monoepoxyvitamin A aldehyde semicarbazone. To about 50mg. of 5,6-monoepoxyvitamin A aldehyde in ethanol, 40mg. of semicarbazide hydrochloride (neutralized with excess of $NaHCO_3$ solution) in aqueous ethanol was added and kept for 30min. on a warm-water bath under N_2. The solution was extracted with diethyl ether and the semicarbazone crystallized from light petroleum containing diethyl ether. The m.p. after three crystallizations was 186–187° (Found: C, 70·06; H, 8·26; N, 11·65; O, 10·0. $C_{21}H_{31}N_3O_2$ requires: C, 70·59; H, 8·68; N, 11·76; O, 8·97%); $\lambda_{max.}$ 358 and 377mμ ($E^{1\%}_{1cm.}$ 1770 and 1560 respectively) (in ethanol), $\lambda_{max.}$ 363 and 383mμ ($E^{1\%}_{1cm.}$ 1510 and 1348 respectively) (in chloroform).

On the addition of ethanolic HCl the $\lambda_{max.}$ of the semicarbazone in ethanol (358 and 377mμ) shifted to 335 and 350mμ. This showed that the 5,6-monoepoxyvitamin A aldehyde semicarbazone was isomerized to 5,8-monoepoxyvitamin A aldehyde semicarbazone.

Reduction of 5,6-monoepoxyvitamin A aldehyde lithium aluminium hydride. $LiAlH_4$ (200mg.) suspended in anhydrous diethyl ether (20ml.) was added slowly at −5° to 5,6-monoepoxyvitamin A aldehyde (60mg.) in dry diethyl ether (25ml.) The solution became colourless within 4min. The excess of $LiAlH_4$ was decomposed by the dropwise addition of cold water, the temperature being kept at 0°. The mixture was extracted with diethyl ether, and the ethereal layer washed and then dried over anhydrous Na_2SO_4. The reduction product, after purification by chromatography on 7%-(v/w)-water-deactivated alumina, was identical with 5,6-monoepoxyvitamin A alcohol in its chromatographic properties and ultraviolet- and infrared-absorption spectra (yield, 40mg.). The compound gave 5,8-monoepoxyvitamin A alcohol on isomerization with ethanolic HCl. This showed that the 5,6-epoxy ring was not affected by the treatment with $LiAlH_4$.

Reduction of 5,6-monoepoxyvitamin A aldehyde in vivo. To a group of six vitamin A-deficient rats, 5,6-monoepoxyvitamin A aldehyde (1mg. in 0·05ml. of deodorized and decolorized groundnut oil/rat/day) was given orally for 10 days. All the rats were killed 3hr. after the last dose and the livers of the rats were ground with anhydrous Na_2SO_4 and fine sand and then extracted with diethyl ether. The diethyl ether was removed under reduced pressure and the oil was dissolved in light petroleum. Chromatographic separation on 7%-(v/w)-water-deactivated alumina showed that the liver lipids contained both esterified and free 5,6-monoepoxyvitamin A. The lipid extract gave a pink colour with $SbCl_3$ reagent that instantaneously changed to yellow, having $\lambda_{max.}$ 460mμ. The $\lambda_{max.}$ at 310 and 325mμ of the lipid extract shifted to 280mμ on the addition of ethanolic HCl, showing isomerization of the 5,6-epoxy group to the 5,8-epoxy group. There was about 30–35% conversion.

Preparation of 5,8-monoepoxyvitamin A aldehyde (VI). Crystalline 5,6-monoepoxyvitamin A aldehyde (200mg.) was dissolved in 20·0ml. of ethanol, and on the addition of 3·0ml. of ethanolic 0·05N-HCl the $\lambda_{max.}$ changed from 365mμ to 330mμ in 3–5min. The reaction was arrested by the addition of dilute aqueous $NaHCO_3$ solution and the product was extracted with diethyl ether. The extract (170mg.) was twice chromatographed on a 7%-(v/w)-water-deactivated alumina (50g.) column (2cm.×25cm.) and the compound was eluted with 10–15% (v/v) diethyl ether in light petroleum. The m.p. of the yellow crystals remained constant at 104–105° after four crystallizations (Found: C, 79·58; H, 9·11; O, 11·31. $C_{20}H_{28}O_2$ requires: C, 79·95; H, 9·39; O. 10·66%).

Preparation of 5,8-monoepoxyvitamin A aldehyde semicarbazone. The semicarbazone was prepared as described above. The m.p. remained constant at 207–208° after four crystallizations (Found: C, 70·81; H, 8·32; N, 11·4; O, 9·47. $C_{21}H_{31}N_3O_2$ requires: C, 70·59; H, 8·68; N, 11·76; O, 8·96%); $\lambda_{max.}$ 334 and 350mμ ($E^{1\%}_{1cm.}$ 1572 and 1387 respectively) (in ethanol), $\lambda_{max.}$ 337 and 353mμ ($E^{1\%}_{1cm.}$ 1494 and 1323 respectively) (in chloroform).

Preparation of 5,8-monoepoxyvitamin A aldehyde 2,4-dinitrophenylhydrazone. 5,8-Monoepoxyvitamin A aldehyde (40mg.) was treated with 30mg. of 2,4-dinitrophenylhydrazine sulphate in ethanol for 10min. at 50° under a stream of N_2. The mixture was kept at −30°, when dark-red crystals appeared. The m.p. remained constant at 218–219° after four crystallizations (Found: C, 64·51; H, 6·69; N, 11·61; O, 17·19. $C_{26}H_{32}N_4O_5$ requires: C, 64·97; H, 6·71; N, 11·67; O, 16·65%); $\lambda_{max.}$ 413 and 325mμ ($E^{1\%}_{1cm.}$ 1502 and 742 respectively) (in ethanol), $\lambda_{max.}$ 415 and 330mμ ($E^{1\%}_{1cm.}$ 1502 and 706 respectively) (in chloroform), $\lambda_{max.}$ 415mμ ($E^{1\%}_{1cm.}$ 1509) (in acetone).

Reduction of 5,8-monoepoxyvitamin A aldehyde by lithium aluminium hydride and in vivo. The procedures followed were similar to those used for 5,6-monoepoxyvitamin A aldehyde.

RESULTS AND DISCUSSION

Studies on the action of per-acids on polyene compounds have shown that a tertiary–tertiary double bond similar to that present in the β-ionone ring is attacked more readily than any other type of double bond (Swern, 1947). Thus it is to be expected that the initial derivative of vitamin A acetate during oxidation with monoperphthalic acid should take place at the 5,6-double bond of the β-ionone ring and have $\lambda_{max.}$ 310mμ, as suggested by Moore (1957). The present investigations clearly demonstrate that oxidation of vitamin A acetate with monoperphthalic acid does give rise to a major fraction with $\lambda_{max.}$ 310mμ (Table 1) that on further purification could be readily crystallized and characterized.

5,6- and 5,8-Monoepoxyvitamin A acetate. The $E^{1\%}_{1cm.}$ values at $\lambda_{max.}$ 310mμ of 5,6-monoepoxyvitamin A acetate in different solvents (Table 2) are much higher than those of crystalline vitamin A acetate (Cama, Collins & Morton, 1951). Spectroscopic data on the chromatographically homogeneous 5,8-monoepoxyvitamin A acetate show that the $E^{1\%}_{1cm.}$ values are lower, both for the ultraviolet-absorption spectra as well as for the antimony trichloride colour test, than those of 5,6-monoepoxyvitamin A acetate.

In the infrared-absorption spectra (Fig. 2), the shoulder at 3145cm.$^{-1}$ present in that of 5,6-monoepoxyvitamin A acetate is absent in that of the 5,8-monoepoxy compound. Henbest, Meakins, Nicholls & Taylor (1957) have suggested that this

Table 1. *Chromatographic separation of the oxidation products of vitamin A acetate with monoperphthalic acid*

The products were chromatographed on 5%-(v/w)-water-deactivated alumina, with light petroleum (b.p. 40–60°) containing various amounts of diethyl ether as the developing solvent. The fractions are listed in the order in which they were eluted from the column.

Fraction	$\lambda_{max.}$ in light petroleum (mμ)	Eluent	Colour with $SbCl_3$ reagent	Yield of fraction from 4·0g. of vitamin A acetate (g.)	Substance
1	325	Light petroleum	Blue	0·455	Unchanged vitamin A acetate
2	310, 325	5–7% (v/v) Ether in light petroleum	Pink, changing to yellow instantaneously	1·026	5,6-Monoepoxyvitamin A acetate
3	280	10–12% (v/v) Ether in light petroleum	Pink, changing to yellow instantaneously	0·125	5,8-Monoepoxyvitamin A acetate
4	310, 325	15–20% (v/v) Ether in light petroleum	Pink, changing to blue, green and finally yellow	0·195	—
5	290, 310, 325	30–40% (v/v) Ether in light petroleum	Faint blue	0·190	—
6	(White decomposed product, difficult to dissolve in light petroleum but soluble in ether)	Ether	Faint blue	2·010	Decomposition products

band may be due to the 5,6-epoxy group in the molecule. Bands shown at 1066 (m), 1083 (w) and 1176cm.$^{-1}$ (w) in the spectra of 5,8-monoepoxyvitamin A acetate are absent in those of 5,6-monoepoxyvitamin A acetate and vitamin A (cf. Farrar, Hamlet, Henbest & Jones, 1952), and thus confirm the furan ring structure in the molecule of 5,8-monoepoxyvitamin A acetate. The strong band at 972cm.$^{-1}$ is due to the out-of-plane hydrogen deformation vibrations of a *trans*

Table 2. *Spectroscopic properties of* 5,6- *and* 5,8-*monoepoxyvitamin A acetate in different solvents*

	5,6-Monoepoxyvitamin A acetate			5,8-Monoepoxyvitamin A acetate	
Solvent	$\lambda_{max.}$ (mμ)	$E^{1\%}_{1cm.}$	$\frac{E_{325m\mu}}{E_{310m\mu}}$ ratio	$\lambda_{max.}$ (mμ)	$E^{1\%}_{1cm.}$
Light petroleum (b.p. 40–60°)	310	1930	0·829	280	1353
	325	1600			
Cyclohexane	313	1900	0·801	281	1353
	325	1522			
Ethanol	310	1807	0·842	278	1378
	324	1522			
Chloroform	316	1750	0·862	283	1136
	327	1510			
$SbCl_3$ colour test	460* (yellow)	1440		460* (yellow)	820

* Initial colour pink, changing to yellow ($\lambda_{max.}$ 460mμ) instantaneously.

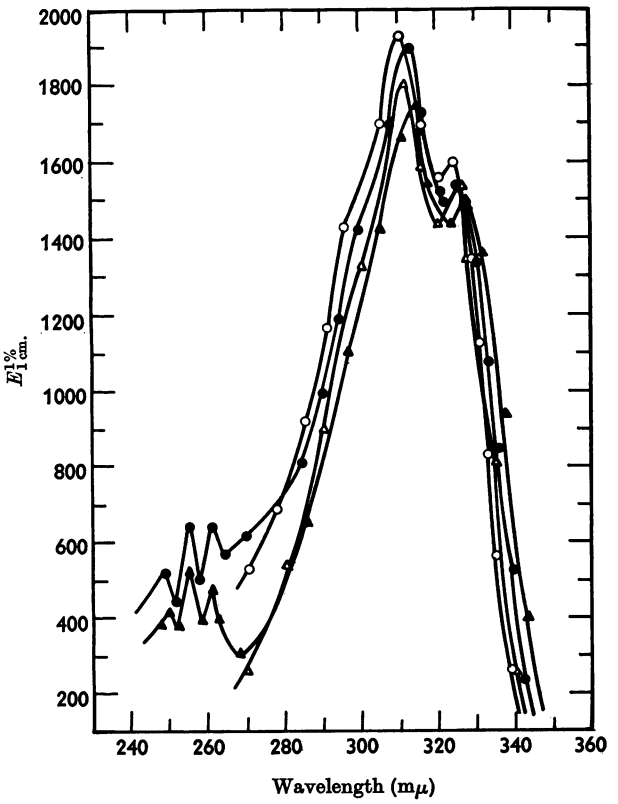


Fig. 1. Ultraviolet-absorption spectra of 5,6-monoepoxyvitamin A acetate in: ○, light petroleum; ●, cyclohexane; △, ethanol; ▲, chloroform.

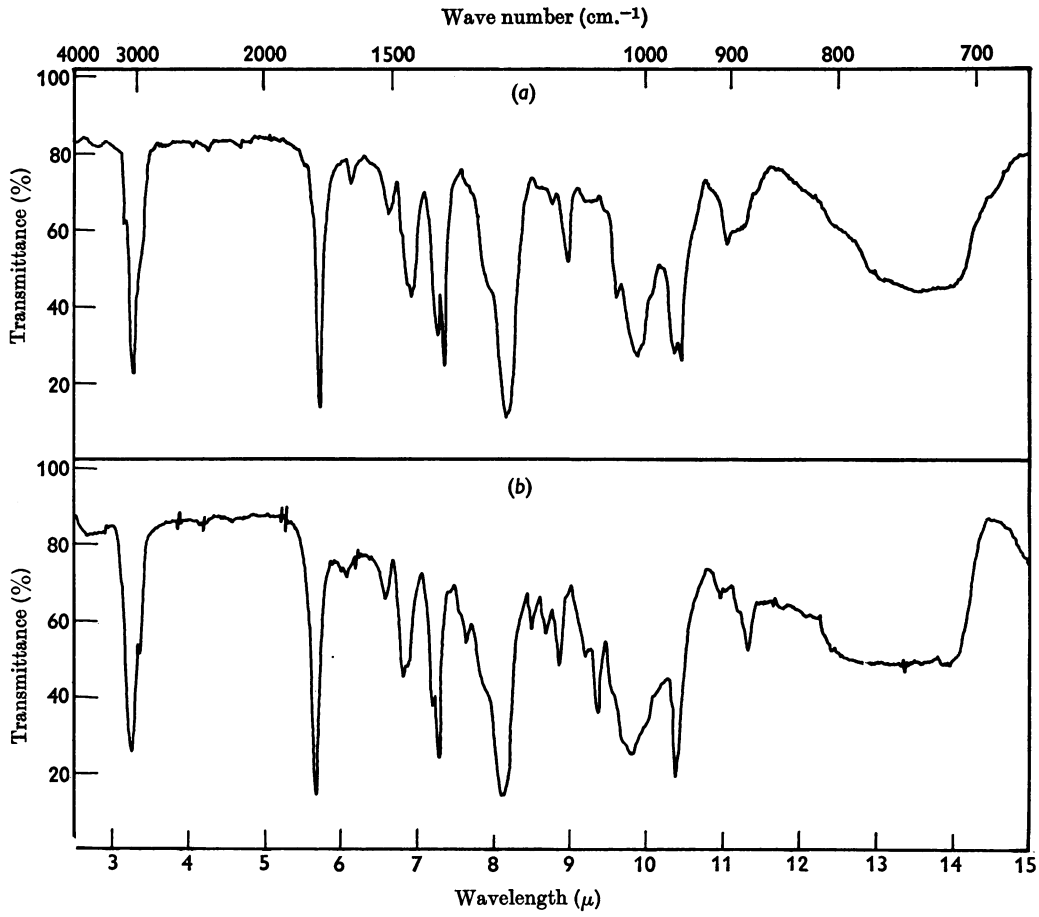


Fig. 2. Infrared-absorption spectra, in carbon tetrachloride, of: (*a*) 5,6-monoepoxyvitamin A acetate; (*b*) 5,8-monoepoxyvitamin A acetate.

Table 3. *Spectroscopic properties of* 5,6- *and* 5,8-*monoepoxyvitamin A alcohol in different solvents*

Solvent	5,6-Monoepoxyvitamin A alcohol			5,8-Monoepoxyvitamin A alcohol	
	$\lambda_{max.}$ (mμ)	$E^{1\%}_{1cm.}$	$\frac{E_{325m\mu}}{E_{310m\mu}}$ ratio	$\lambda_{max.}$ (mμ)	$E^{1\%}_{1cm.}$
Light petroleum (b.p. 40–60°)	310	2413	0·855	279	1775
	325	2065			
Cyclohexane	312	2335	0·847	281	1508
	327	1978			
Ethanol	310	2422	0·856	278	1768
	324	2075			
Chloroform	315	2249	0·856	284	1462
	329	1925			
$SbCl_3$ colour test	465* (yellow)	1272		465† (yellow)	850

* Initial colour pink, changing to blue, green and finally yellow ($\lambda_{max.}$ 465mμ) instantaneously.
† Initial colour pink, changing to yellow ($\lambda_{max.}$ 465mμ) instantaneously.

–CH=CH– conjugated system, and is also present in the spectra of vitamin A_1 aldehyde and vitamin A_2 aldehyde (Farrar *et al.* 1952).

5,6- *and* 5,8-*Monoepoxyvitamin A alcohol.* The $E^{1\%}_{1\,cm.}$ values of 5,6- and 5,8-monoepoxyvitamin A alcohol (Table 3) are higher than those of the corresponding acetates. The subsidiary peak at 325mμ in the ultraviolet-absorption spectra of 5,6-monoepoxyvitamin A acetate and alcohol is an inherent property of the compounds and is not due to any contamination, but its presence is difficult to explain. The hypsochromic shift of 15–20mμ from the $\lambda_{max.}$ of vitamin A (328mμ) to that of 5,6-monoepoxyvitamin A acetate and alcohol indicates the loss of one conjugated double bond from the molecule. The ultraviolet-absorption spectra of 5,8-monoepoxyvitamin A alcohol are shown in Fig. 3.

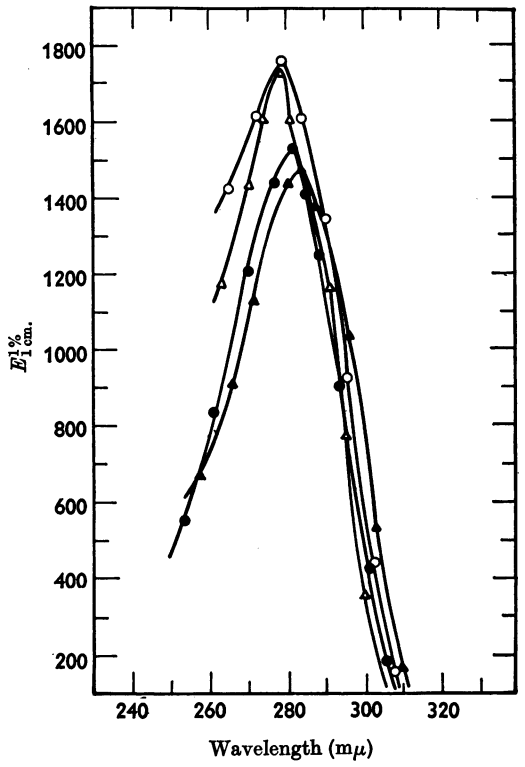


Fig. 3. Ultraviolet-absorption spectra of 5,8-monoepoxyvitamin A alcohol in: ○, light petroleum; ●, cyclohexane; △, ethanol; ▲, chloroform.

The infrared-absorption spectra of 5,6- and 5,8-monoepoxyvitamin A alcohol showed behaviour identical with those of the corresponding acetates, except for the presence of the 3704 (m) and 3650cm.$^{-1}$ (w) bands due to O–H stretching vibrations, instead of the acetoxy group.

5,6- *and* 5,8-*Monoepoxyvitamin A aldehyde.* The $E^{1\%}_{1\,cm.}$ values of 5,6- and 5,8-monoepoxyvitamin A aldehyde are comparatively higher than that of vitamin A aldehyde (Ball *et al.* 1948), as evident from Table 4 and Figs. 4 and 5. Further, 5,6-monoepoxyvitamin A aldehyde does not have a characteristic subsidiary peak, unlike its corresponding acetate or the alcohol. The shift of 30mμ observed in the ultraviolet-absorption spectra of 5,6-monoepoxyvitamin A acetate and alcohol with concomitant lowering of extinction values clearly indicates the isomerization of 5,6- to 5,8-monoepoxides (cf. Karrer & Jucker, 1950; Jungalwala & Cama, 1962). The shift in the ultraviolet-absorption spectrum of 5,6-monoepoxyvitamin A aldehyde to its furanoid form is only a little greater than 30mμ but the extinction values are not markedly affected. The difference of about 45–50mμ between $\lambda_{max.}$ of vitamin A acetate or alcohol (328mμ) to that of 5,8-monoepoxyvitamin A acetate or alcohol (280mμ), and that of vitamin A aldehyde (370mμ) to that of 5,8-monoepoxyvitamin A aldehyde

Table 4. *Spectroscopic properties of* 5,6- *and* 5,8-*monoepoxyvitamin A aldehyde in different solvents*

Solvent	5,6-Monoepoxyvitamin A aldehyde		5,8-Monoepoxyvitamin A aldehyde	
	$\lambda_{max.}$ (mμ)	$E^{1\%}_{1\,cm.}$	$\lambda_{max.}$ (mμ)	$E^{1\%}_{1\,cm.}$
Light petroleum (b.p. 40–60°)	352	1811	317	1725
			331	1614
Cyclohexane	353	1587	319	1651
			333	1540
Ethanol	365	1511	331	1460
Chloroform	367	1491	337	1491
$SbCl_3$ colour test	440* (yellow)	1700	440* (yellow)	1031
	490*	1240	490*	940

* Initial colour greenish-blue, changing to yellow ($\lambda_{max.}$ 440 and 490mμ) instantaneously.

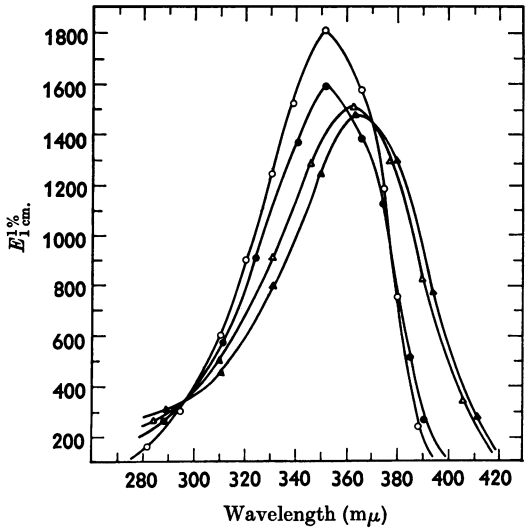


Fig. 4. Ultraviolet-absorption spectra of 5,6-monoepoxyvitamin A aldehyde in: ○, light petroleum; ●, cyclohexane; △, ethanol; ▲, chloroform.

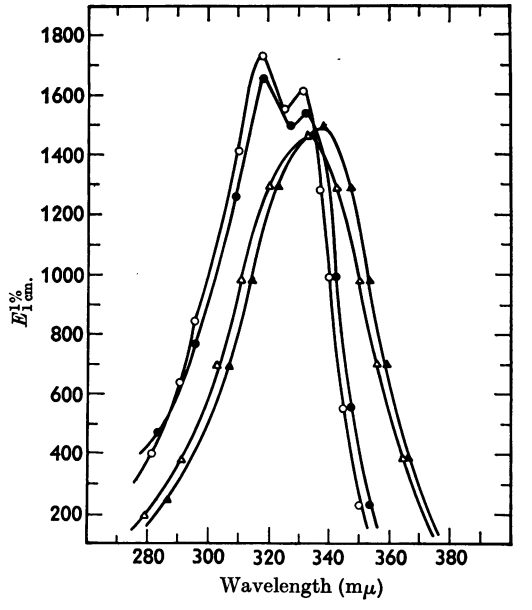


Fig. 5. Ultraviolet-absorption spectra of 5,8-monoepoxyvitamin A aldehyde in: ○, light petroleum; ●, cyclohexane; △, ethanol; ▲, chloroform.

(320mμ), indicates the loss of two conjugated double bonds in the furanoid group of compounds (Scheme 1).

The antimony trichloride colour test on the 5,6- and 5,8-monoepoxyvitamin A acetate, alcohol and aldehyde does not agree with the expected shift in the $\lambda_{max.}$ values as observed with the vitamin A_1 and A_2 groups of compounds. Further, all these epoxy compounds do not give a characteristic blue colour, but instead give an initial pink colour that instantaneously changes to stable yellow colour which could be used for their quantitative estimation (Lakshmanan, Jungalwala & Cama, 1965).

The infrared-absorption spectra (Fig. 6) of 5,6- and 5,8-monoepoxyvitamin A aldehyde showed strong bands at 1669 and 1667cm.$^{-1}$ respectively, which could be attributed to the $\alpha\beta$-unsaturated C=O group in the molecule. The presence of the aldehyde group is confirmed by the appearance of bands at 2800 and 2849cm.$^{-1}$ in the spectrum of 5,6-monoepoxyvitamin A aldehyde and at 2841cm.$^{-1}$ in that of 5,8-monoepoxyvitamin A aldehyde. These bands probably arise from the valence vibrations of H atom attached to the C–O group (Pozefsky & Coggeshall, 1951; Pinchas, 1957). In other respects the infrared-absorption bands of these two compounds showed behaviour similar to that of their corresponding acetate and alcohol.

The nuclear-magnetic-resonance spectrum of 5,6-monoepoxyvitamin A aldehyde (Fig. 7) supports the assigned structure (Scheme 1). The integration curve shows that all the 28 protons are accounted for. In the lower field, 5·7–7·2 p.p.m. (2·8–4·3τ), all the seven protons on the double bonds are available. Owing to complexities arising from spin-coupling, it is difficult to correlate these signals with individual protons. The spectrum clearly shows that carbon atoms carrying the oxide bridge do not carry any protons [absence of any signal between 3·0 and 5·5p.p.m. (4·5 to 6·0τ); Fig. 6 is therefore condensed], as expected from the 5,6-epoxy structure (cf. Kofler & Rubin, 1960). The bands at 2·3p.p.m. (7·7τ) and 1·93p.p.m. (8·07τ) are respectively assigned to the methyl protons at C-9 and C-13. Protons from the *gem*-dimethyl group at C-1 come at 1·12p.p.m. (8·88τ) as a sharp band. The protons of the methyl group at C-5 show a shift to a higher field [0·9p.p.m. (9·1τ)] as they probably come under the magnetic effect of the 7,8-double bond. This can only happen if the methyl group at C-5 and the olefinic side chain at C-6 are *trans* to each other, a relationship consistent with the 5,6-epoxy structure. The six methylene (ring) protons come as ill-differentiated signals covering the field from 1·0 to 2·0p.p.m. (8·0–9·0τ). The nuclear-magnetic-resonance spectrum of 5,8-monoepoxyvitamin A aldehyde was also taken but, as mentioned above, the compound was unstable and the spectrum was not satisfactory. Nonetheless, there were two peaks at 4·82p.p.m. (5·18τ) and 4·95p.p.m. (5·05τ) that could be

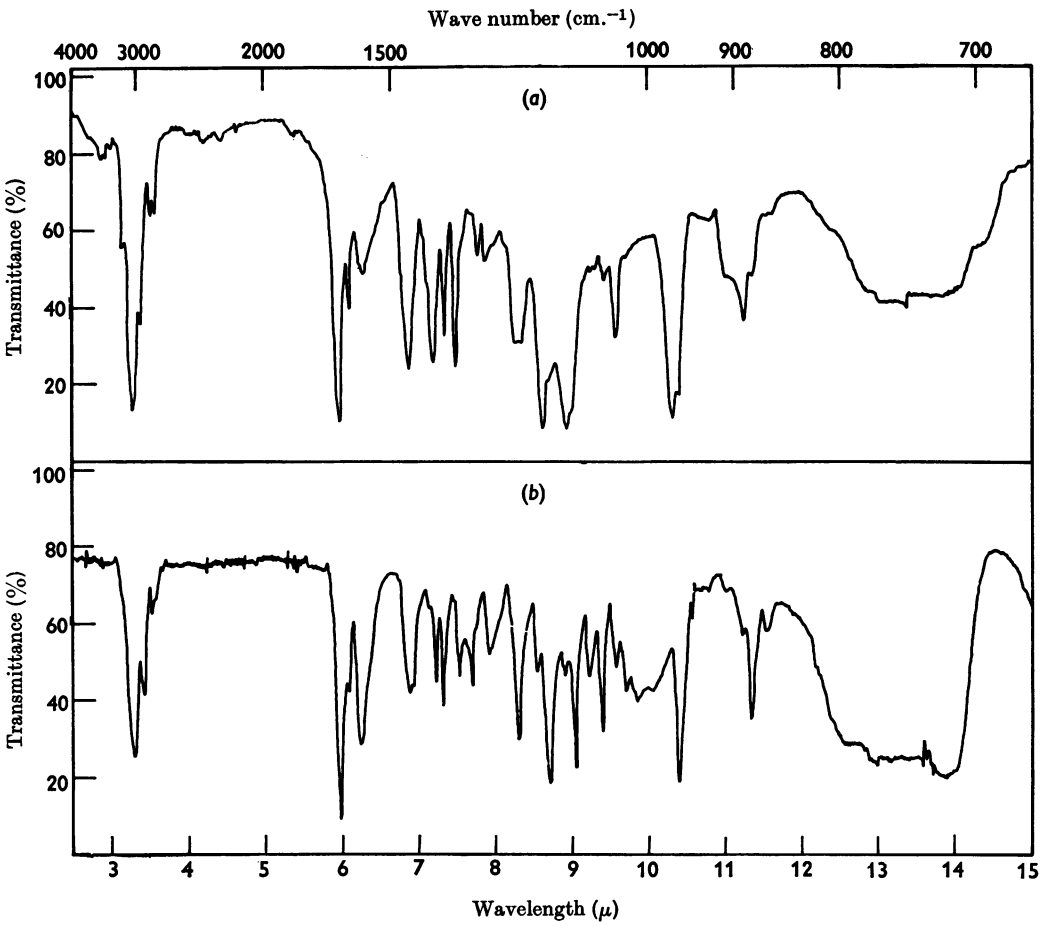


Fig. 6. Infrared-absorption spectra, in carbon tetrachloride, of: (*a*) 5,6-monoepoxyvitamin A aldehyde; (*b*) 5,8-monoepoxyvitamin A aldehyde.

assigned to the two protons at positions 7 and 8. This is consistent with the structure of 5,8-monoepoxyvitamin A aldehyde (Scheme 1).

The semicarbazones of 5,6- and 5,8-monoepoxyvitamin A aldehyde and the phenylhydrazone of 5,8-monoepoxyvitamin A aldehyde gave satisfactory analyses and their spectra in different solvents agreed closely with expected shifts in $\lambda_{max.}$, consistent with similar derivatives of vitamin A_1 aldehyde (Ball *et al.* 1948) and vitamin A_2 aldehyde (Cama *et al.* 1952).

The reduction of 5,6-monoepoxyvitamin A aldehyde with lithium aluminium hydride or *in vivo* yielded 5,6-monoepoxyvitamin A alcohol, with a characteristic absorption spectra ($\lambda_{max.}$ at 310 and 325 mμ) (Fig. 1). Further, the $E_{325m\mu}/E_{310m\mu}$ ratio was the same as that observed for 5,6-monoepoxyvitamin A acetate and alcohol (Tables 2 and 3), confirming the inherent property of the ultraviolet-absorption characteristics of both the 5,6-monoepoxy compounds. Similarly, the reduction of 5,8-monoepoxyvitamin A aldehyde, both *in vitro* and *in vivo*, gave 5,8-monoepoxyvitamin A alcohol, with an identical ultraviolet-absorption spectrum (Fig. 3).

General remarks. It has been shown that the action of monoperphthalic acid on vitamin A acetate, rather than on the alcohol, gives 5,6-monoepoxyvitamin A alcohol in good yield, and the products were well characterized. From the elementary analyses, ultraviolet- and infrared-absorption spectra, nuclear-magnetic-resonance spectra and studies on the derivatives and the conversions *in vitro* and *in vivo*, the structure of the most stable compound, 5,6-monoepoxyvitamin A aldehyde (V), was conclusively established, and this gives considerable support to the other structures proposed (Scheme 1). The next paper

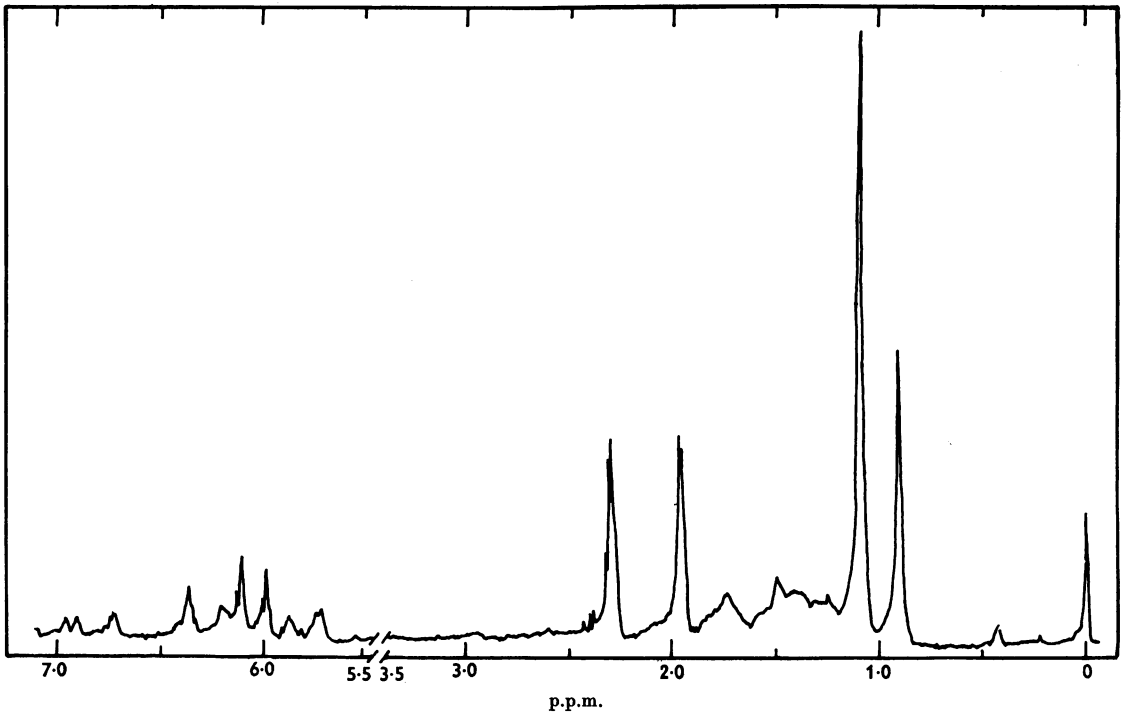


Fig. 7. Nuclear-magnetic-resonance spectrum of 5,6-monoepoxyvitamin A aldehyde.

(Lakshmanan *et al.* 1965) shows the role of 5,6-monoepoxyvitamin A aldehyde in metabolic studies.

We thank Dr Otto Isler, Hoffmann–La Roche, Basle, Switzerland, for a generous gift of crystalline synthetic vitamin A, and Dr P. K. Bhattacharya, National Chemical Laboratory, Poona, India, for kindly arranging to take the nuclear-magnetic-resonance spectra and for their interpretation. The financial assistance of the C.S.I.R. (India) is gratefully acknowledged.

REFERENCES

Bagnall, H. H. & Stock, F. G. (1952). *Analyst*, **77**, 356.
Ball, S., Goodwin, T. W. & Morton, R. A. (1948). *Biochem. J.* **42**, 516.
Böhme, H. (1955). *Org. Synth.* **3**, 619.
Bolomey, R. A. (1947). *J. biol. Chem.* **169**, 331.
Cama, H. R., Collins, F. D. & Morton, R. A. (1951). *Biochem. J.* **50**, 48.
Cama, H. R., Dalvi, P. D., Morton, R. A., Salah, M. K., Steinberg, G. R. & Stubbs, A. L. (1952). *Biochem. J.* **52**, 535.
Embree, N. D. & Shantz, E. M. (1943). *J. Amer. chem. Soc.* **65**, 906.
Euler, H. von, Karrer, P. & Zubrys, A. (1934). *Helv. chim. acta*, **17**, 24.
Farrar, K. R., Hamlet, J. C., Henbest, H. B. & Jones, E. R. H. (1952). *J. chem. Soc.* p. 2657.
Groot, E. H. (1949). *Rec. Trav. chim. Pays-Bas*, **68**, 185.
Halpern, G. R. (1946). *Industr. Engng Chem.* (*Anal. Ed.*), **18**, 621.
Henbest, H. B., Meakins, G. D., Nicholls, B. & Taylor, K. J. (1957). *J. chem. Soc.* p. 1459.
Jungalwala, F. B. & Cama, H. R. (1962). *Biochem. J.* **85**, 1.
Karrer, P. & Jucker, E. (1945). *Helv. chim. acta*, **28**, 717.
Karrer, P. & Jucker, E. (1947). *Helv. chim. acta*, **30**, 559.
Karrer, P. & Jucker, E. (1950). *Carotenoids*. Translated into English by Braude, E. A. London: Elsevier Publishing Co.
Kofler, M. & Rubin, S. H. (1960). *Vitam. & Horm.* **18**, 315
Lakshmanan, M. R., Jungalwala, F. B. & Cama, H. R. (1965). *Biochem. J.* **95**, 27.
LePage, G. A. & Pett, L. B. (1941). *J. biol. Chem.* **141**, 747.
Meunier, P. & Jouanneteau, J. (1948). *Bull. Soc. Chim. biol., Paris*, **30**, 185.
Milas, N. A. (1947). *Vitam. & Horm.* **5**, 39.
Moore, T. (1957). *Vitamin A*, pp. 120–122. Amsterdam: Elsevier Publishing Co.
Morton, R. A. & Goodwin, T. W. (1944). *Nature, Lond.*, **153**, 405.
Pinchas, S. (1957). *Analyt. Chem.* **29**, 334.
Pozefsky, A. & Coggeshall, N. D. (1951). *Analyt. Chem.* **23**, 1611.
Pritchard, H., Wilkinson, H., Edisbury, J. R. & Morton, R. A. (1937). *Biochem. J.* **31**, 258.
Royals, E. E. & Harrell, L. L., jun. (1955). *J. Amer. chem. Soc.* **77**, 3405.
Swern, D. E. (1947). *J. Amer. chem. Soc.* **69**, 1692.