443. *The Determination of Oxiran-oxygen, with Special Reference to its Application to the Study of Autoxidation.*

By George King.

A quantitative study of the absorption of hydrogen chloride from organic solvents by certain 1 : 2-epoxides and olefinic ketones has been made. A simple method of determining epoxides based on this principle, without strictly anhydrous conditions, has been further developed and critically examined. It has been shown that the presence of a carbonyl group may interfere with the quantitative absorption of hydrogen chloride by an epoxide, and α-olefinic ketones may also absorb hydrogen chloride to a variable extent.

The application of the method to the investigation of the autoxidation of oleic acid has been exemplified. In this connexion, the absorption of ultra-violet light in the region of λ 220 mμ. has been used to estimate the concentration of κ- and η-keto-oleic (10- and 7-ketoheptadec-8-ene-1-carboxylic) acids and hence to apply suitable corrections to the oxiran-oxygen values.

trans-11-Ketoheptadec-9-ene-1-carboxylic acid has been further studied, and its ultra-violet absorption spectrum recorded.

It has generally been assumed that the absorption of hydrogen chloride from organic solvents by 1 : 2-epoxides is more or less quantitative (Nicolet and Poulter, *J. Amer. Chem. Soc.*, 1930, **52**, 1186; Swern *et al.*, *Analyt. Chem.*, 1947, **19**, 414). In a preliminary communication

(*Nature*, 1949, **164**, 706) the author described a convenient method of determining epoxides, based on the absorption of hydrogen chloride from a solution of hydrochloric acid in dioxan during ten minutes. The results obtained with simple epoxides, such as oleic and elaidic acid oxides, were nearly quantitative and compared favourably with those obtained by earlier methods, involving strictly anhydrous conditions and a much longer time of contact. It was subsequently shown, however, that the presence of neighbouring reactive groups, such as carbonyl, as in λ-ketoelaidic acid oxide, may interfere with the absorption; further, that certain unsaturated ketones, more especially those of the type $R\cdot CO\cdot CH{:}CHR'$, also absorb hydrogen chloride (King, *J.*, 1950, 2897), a property which assumes particular importance when the method is applied to the autoxidation products of unsaturated fatty acids. These defects appear to be inherent in all methods based on the absorption of hydrogen chloride from organic solvents, whether anhydrous or not. Ross's method (*J.*, 1950, 2257), based on the development of alkalinity when an epoxide is boiled with an aqueous-acetone solution of sodium thiosulphate, is inapplicable to the determination of long-chain epoxides of the type under investigation.

Table I records the oxiran-oxygen values found for a variety of 1 : 2-epoxides, and the absorption of hydrogen chloride by certain olefinic ketones is given in Table II. Of the epoxides studied, λ-ketoelaidic acid oxide is exceptional in absorbing only 72% of the theoretical quantity of hydrogen chloride, a result which may be attributed to the influence of the carbonyl group. The absorption by unsaturated ketones under these conditions is very variable and is sometimes mainly dependent on the time of contact (see, *e.g.*, mesityl oxide, Table II). The α-olefinic ketone *trans*-11-ketoheptadec-9-ene-1-carboxylic acid (I) absorbs hydrogen chloride rapidly and almost quantitatively. This compound is closely analogous to the structurally isomeric κ- (II) and η-keto-oleic acids (III) and the corresponding ketoelaidic acids, which appear in significant amounts during the autoxidation of oleic and elaidic acids at 50—60° (Ellis, *Biochem. J.*, 1950, **46**, 129; *J.*, 1950, 9).

TABLE I.

	Oxiran-oxygen (%).		
	HCl–dioxan method.	Method of Swern *et al.*	Theory.
Oleic acid oxide	5·19	5·18	5·36
Elaidic acid oxide	5·17	5·19	5·36
Ethyl oleate oxide	4·66	4·62	4·90
Ricinelaidic acid oxide	4·92	5·10	5·09
λ-Ketoelaidic acid oxide	3·68	3·58	5·12
Epichlorohydrin	16·76	—	17·30
Butadiene monoxide	21·60	21·50	22·8

TABLE II.

	Mols. of HCl absorbed per mol. of substance.	
	HCl–dioxan method.	Method of Swern *et al.*
λ-Keto-oleic acid	0·10	0·13
λ-Ketoelaidic acid	0·01	—
κλ-Diketo-oleic acid	0·15	0·14
Mesityl oxide	0·22	0·65
	0·49 (2 hours)	
	0·60 (3 hours)	
trans-11-Ketoheptadec-9-ene-1-carboxylic acid	0·90	0·93
Benzylideneacetone	Nil	—

$R\cdot CO\cdot CH{:}CH\cdot[CH_2]_2\cdot R'$ (I.)

$R\cdot CH_2\cdot CO\cdot CH{:}CH\cdot CH_2R'$ (II.)

$R\cdot[CH_2]_2\cdot CH{:}CH\cdot CO\cdot R'$ (III.)

$R\cdot CO\cdot CH_2\cdot CH{:}CH\cdot CH_2R'$ (IV.)

($R = CH_3\cdot[CH_2]_5$; $R' = [CH_2]_6\cdot CO_2H$.)

Although (II) and (III) have not yet been isolated, they are known to give rise on alkaline hydrolysis to the volatile nonan-2-one and nonaldehyde, respectively, whereby their concentrations in autoxidised oils may be estimated with reasonable accuracy. By analogy with (I), it is highly probable that these keto-acids absorb some 90% of the theoretical amount of hydrogen chloride required per double bond under the conditions used for the determination of oxiran-oxygen, and the latter values for the total autoxidation products of oleic or elaidic acid must be corrected accordingly.

The acid (I) has now been studied in further detail. Its ultra-violet absorption spectrum (Fig. 1) exhibits a band of high intensity ($\varepsilon_{max.}$ 16,000) at 224 mμ. and a weak band ($\varepsilon_{max.}$ 53) at 317 mμ., thus closely resembling that of κλ-diketo-oleic (*cis*-10 : 11-diketoheptadec-8-ene-1-carboxylic) acid (King, *J.*, *loc. cit.*). A value of $\varepsilon_{max.}$ 16,000 in the region of 220 mμ. appears to be consistently associated with the group •CO•CH:CH•, and it may be assumed that the spectra of the analogous acids (II) and (III) have bands of the same intensity in this region. The absorption spectrum of oleic acid, autoxidised at 70—80° in the presence of a cobalt catalyst, also exhibits a peak at *ca.* 220 mμ., and the increase in the extinction at this wave-length, which runs closely parallel to the amount of volatile oil obtainable by alkaline hydrolysis, affords an alternative, and probably more accurate, basis for the determination of the total κ- and η-keto-acid content.

FIG. 1.

Absorption spectrum of trans-11-*ketoheptadec*-9-*ene*-1-*carboxylic acid.*

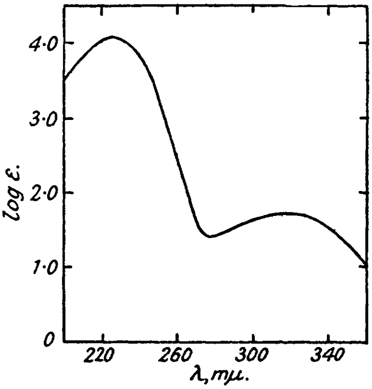
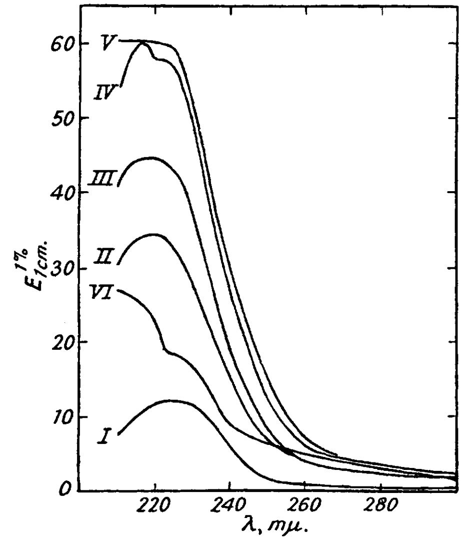


FIG. 2.



I. Original oleic acid. *IV. After* 9 *hours' autoxiation.*
II. After 3 *hours' autoxidation.* *V.* ,, 12 ,,
III. ,, 6 ,, *VI.* ,, 31 ,,

A typical example of the application of the spectrophotometric method to the assessment of olefinic keto-acid formation during the catalytic autoxidation of oleic acid and the influence of such acids on the observed oxiran-oxygen values is summarised in Table III and Fig. 2. Oxygen was bubbled through oleic acid containing 0·5% of cobalt at 77—78°. The total concentration of keto-acids (II and III) was calculated from the increase in the absorption at 224 mμ. on the assumption that the pure acids have $\varepsilon_{max.}$ 16,000 at or very near this wave-length, and it was further assumed that they absorb 90% of the theoretical amount of hydrogen chloride, equivalent to an apparent oxiran-value of 4·85%. The final verification of these assumptions necessarily awaits the isolation of (II) and (III) in a pure state; nevertheless, remarkably good

TABLE III.

Autoxidation at 77—78° *of oleic acid containing* 0·5% *of cobalt.*

Time (hours)		0	3	6	9	12	31
Equiv. wt.		282	287	284	266	246	197
I Val. (Wijs, ¾ hour)		85	70	56	39	24	6
Active oxygen (Lea), %		Trace	0·03	0·03	0·04	0·04	0·05
Apparent oxiran-O, %		0·18	0·71	1·03	1·45	1·63	0·77
$E^{1\%}_{1\,cm.}$ cm. at λ 224 mμ.		12	34	44	58	60	19
κ- and η-Keto-oleic acids, %		—	4·2	6·1	8·7	9·1	1·3
Oxiran-O (corr.), %		—	0·51	0·73	1·03	1·19	0·71
Elaidic acid oxide, %		—	9·5	13·6	19·2	22·2	13·3
Volatile hydrolysis products,* %,	found	—	1·6	2·9	3·9	3·7	0·6
	calc.	—	2·0	2·9	4·2	4·4	0·6

* Nonan-2-one, nonanal, and nonanoic acid.

correspondence was observed between the total steam-volatile hydrolysis products, consisting of nonan-2-one, nonaldehyde, and a little nonanoic acid arising from atmospheric oxidation, and the calculated amounts. The progressive increase in the keto-acid concentration up to a maximum of some 9% after 12 hours' oxidation was also closely paralleled by the intensity of the purple colour which developed on treatment of the autoxidised oil with 3 : 5-dinitrobenzoic acid in ethanolic sodium hydroxide, but was insufficient to cause a perceptible peak in the ultra-violet light absorption at 317 mμ. The maximum content of epoxide in terms of elaidic acid oxide was 22%, which accords well with the 16—20% actually isolated by Ellis (*Biochem. J.*, 1936, **30**, 753) after similar autoxidations.

The marked fall in the amounts of keto-acid and epoxide on prolonged oxidation was presumably caused by chain scission at the double bond, and to ester formation resulting from union of the epoxide with itself or with acid cleavage products (Ellis, *Biochem. J.*, 1950, **46**, 129). The concentration of peroxides was low throughout. No κλ-diketo-oleic acid (King, *J.*, *loc. cit.*) could be detected among the products, and it may be inferred that little or no λ-keto-oleic acid is formed under these conditions, since it undergoes autoxidation to the former very rapidly.

It may be remarked that the absorption of ultra-violet light below 240 mμ. by ordinary commercial samples of "oleic acid" is not usually due to the presence of keto-acids. Such impure samples commonly have $E^{1\%}_{1\,\mathrm{cm.}}$ values of about 25 at 220—225 mμ., yet contain no measurable amount of keto-oleic acids: the dinitrobenzoic acid test is virtually negative and no volatile oil is obtained when the acid is boiled with dilute alkali.

The absorption of hydrogen bromide from dioxan or ether solution by (I) affords almost exclusively 9-bromo-11-ketoheptadecane-1-carboxylic acid, m. p. 70·5°, which also results from the addition of hydrogen bromide to λ-keto-oleic (or elaidic) acid (IV) and from which (I) is readily regenerated by the action of cold aqueous alkali. Similarly, (I) combines with hydrogen chloride to form an adduct, m. p. 70°, which, by analogy with the bromo-compound, is probably 9-chloro-11-ketoheptadecane-1-carboxylic acid. Oxidation of (I) with alkaline permanganate at 100° gives *n*-heptanoic and sebacic acids.

EXPERIMENTAL.

Determination of Oxiran-oxygen.—The method used was essentially that already described (*Nature*, *loc. cit.*), but greater accuracy was achieved by using double quantities, *i.e.*, 0·2 g. of epoxide-containing material and 20 ml. of the dioxan–hydrochloric acid reagent, followed after 10—15 minutes by 20 ml. of neutral ethanol. The epoxide was weighed out in a conical flask of 150-ml. capacity, carrying a ground-glass stopper, and the reagent was delivered from a burette to avoid inhaling the vapour unnecessarily. In most cases the method of Swern *et al.* (*loc. cit.*) was also used for comparison.

Autoxidation of Oleic Acid.—Oleic acid was purified by the method of Brown and Shinowara (*J. Amer. Chem. Soc.*, 1937, **59**, 6) and had $E^{1\%}_{1\,\mathrm{cm.}}$ 12 at 224 mμ. Oxygen was bubbled at the rate of 30 ml. per minute by way of a sintered-glass filter-stick through the acid (16 ml.) at 77—78°, finely powdered cobalt hydroxide (0·5% as Co) being added as a catalyst. Samples (2 ml.) of the oil were withdrawn at intervals for analysis. After 9 hours or more the cooled product deposited some unidentified crystalline material.

κ- and η-Keto-oleic Acids (II and III).—The total concentration of these acids was calculated from the increase in the value of $E^{1\%}_{1\,\mathrm{cm.}}$ at 224 mμ. The pure keto-acids being assumed to have $E^{1\%}_{1\,\mathrm{cm.}}$ 540 ($\varepsilon_{\mathrm{max.}}$ 16,000) at this wave-length, their percentage concentration is given by $(E^{1\%}_{1\,\mathrm{cm.}} - 12)/5{\cdot}28$. All absorption spectra were examined in ethanol with a Unicam Model S.P. 500 Spectrophotometer.

Correction of Oxiran-oxygen Values.—By analogy with (I), the keto-acids (II) and (III) were assumed to absorb 0·9 mol. of hydrogen chloride under the above conditions, corresponding to an apparent oxiran-oxygen value of 4·85%. Thus, of the total oxiran-oxygen value of 1·63% observed after 12 hours' autoxidation (Table III), 0·44% was contributed by the keto-acids (9·1%), leaving a corrected value of 1·19%, equivalent to a content of 22·2% of elaidic acid oxide.

Volatile Hydrolysis Products.—The autoxidised oil (*ca.* 1 g.) in 0·1N-sodium hydroxide (100 ml.) was very slowly distilled, and the insoluble steam-volatile products were quantitatively collected in a special trap (Ellis, *Biochem. J.*, *loc. cit.*). It was confirmed that this volatile oil consisted largely of nonan-2-one (semicarbazone, m. p. 117—118° not depressed by an authentic specimen) with a small proportion of nonanal (crude 2 : 4-dinitrophenylhydrazone, m. p. 94° raised to 96—97° by an authentic specimen) and some 11—12% of nonanoic acid arising from atmospheric oxidation of the latter. It gave a weak Schiff's test and the proportion of nonanal was insufficient to give any crystalline deposit with dimedone in 50% ethanol.

trans-11-*Ketoheptadec*-9-*ene*-1-*carboxylic Acid* (I).—This acid was prepared by the following modification of Ellis's method (*J.*, *loc. cit.*). λ-Ketoelaidic acid (8·4 g.), m. p. 72·5°, in dioxan (250 ml.) was shaken with concentrated hydrobromic acid (20 ml.; *d* 1·7) at intervals during 1 hour. The diluted and cooled solution deposited 9-bromo-11-ketoheptadecane-1-carboxylic acid, which crystallised from *n*-hexane in needles (9·6 g.), m. p. 70·5° (Found: C, 57·6; H, 8·9; Br, 21·0. Calc. for $C_{18}H_{33}O_3Br$: C, 57·3; H, 8·8; Br, 21·2%). The product was dissolved in 0·25N-sodium hydroxide (450 ml.) and kept for 1 hour at room temperature. Acidification with acetic acid then afforded (I), which crystallised from acetic acid in thin hexagonal plates (6·1 g.), m. p. 50·5°, freely soluble in warm light petroleum, but sparingly soluble at 0° [Found: C, 72·9; H, 10·9%; equiv., 296; I.V. (Wijs, 45 minutes), 83·4. Calc. for $C_{18}H_{32}O_3$: C, 72·9; H, 10·9%; equiv., 296; I.V., 86]. The *semicarbazone* separated from ethanol in hexagonal tablets or prisms, m. p. 141·5° (Found: C, 64·7; H, 9·7; N, 12·0. $C_{19}H_{35}O_3N_3$ requires C, 64·5; H, 10·0; N, 11·9%).

The acid (I) absorbed 90% of the theoretical amount of hydrogen chloride required for 1 double bond under the above conditions, equivalent to an apparent oxiran-oxygen value of 4·85%. It gave a deep purple colour with 3 : 5-dinitrobenzoic acid in ethanolic sodium hydroxide, by which as little as 10 μg. could be detected. Its absorption spectrum in ethanol had $\varepsilon_{max.}$ 16,000 at 224 mμ. and a feeble maximum $\varepsilon_{max.}$ 53 at 317 mμ. Hydrolysis of the acid with 0·05N-sodium hydroxide afforded a 38% yield (theory, 43%) of octan-2-one (2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 68·5°).

Oxidation of trans-11-*Ketoheptadec*-9-*ene*-1-*carboxylic Acid* (I).—The acid (0·75 g.) was dissolved in 0·5% aqueous sodium hydroxide (20 ml.) and heated with potassium permanganate (1·7 g.) in water (20 ml.) at 100° for 10 minutes. The cooled solution was acidified with concentrated hydrochloric acid, saturated aqueous sodium metabisulphite (1 ml.) was added, and the whole diluted to about 120 ml. with water. Distillation afforded *n*-heptanoic acid (0·24 g.) (Found: equiv., 128. Calc. for $C_7H_{14}O_2$: equiv., 130), whose zinc salt crystallised from ethanol in rectangular plates, m. p. and mixed m. p. 136°. The residual solution, cooled at 0°, deposited sebacic acid (0·37 g.), which was dissolved in ether and reprecipitated by addition of 2 volumes of *n*-hexane; it then crystallised from water in thick rhombic and hexagonal plates, m. p. and mixed m. p. 132° (Found: equiv., 102. Calc. for $C_{10}H_{18}O_4$: equiv., 101).

9-*Chloro*-11-*ketoheptadecane*-1-*carboxylic Acid*.—The acid (I) (0·54 g.) in dioxan (25 ml.) was shaken with concentrated hydrochloric acid (1 ml.) in a stoppered flask and left for 1 hour. The diluted and cooled solution deposited 9-*chloro*-11-*ketoheptadecane*-1-*carboxylic acid* (0·58 g.), which crystallised from *n*-hexane in needles, m. p. 70° (Found: C, 64·7; H, 9·9; Cl, 10·5%; equiv., 327. $C_{18}H_{33}O_3Cl$ requires C, 64·9; H, 10·0; Cl, 10·7%; equiv., 333). This compound has already been prepared from λ-keto-oleic acid by Dr. G. W. Ellis (private communication).

Other Materials.—λ-Keto-oleic acid, m. p. 40°, and λ-ketoelaidic acid, m. p. 73° (Ellis, *loc. cit.*); oleic acid oxide, m. p. 59·5° (King, *J.*, 1942, 390); elaidic acid oxide, m. p. 55·5°, and ethyl oleate oxide, m. p. 21° (King, *J.*, 1948, 1820); ricinelaidic acid oxide, m. p. 65—66°, λ-ketoelaidic acid oxide, m. p. 84°, and κλ-diketo-oleic acid (King, *J.*, 1950, 2897); mesityl oxide, b. p. 130—132°, purified by washing with aqueous alkali and fractionation.

The author is indebted to Imperial Chemical Industries Limited for a sample of butadiene monoxide.

ST. MARY'S HOSPITAL MEDICAL SCHOOL, LONDON, W.2.

[*Received, April* 9*th*, 1951.]