much higher than normal: in the series of animals studied, concentrations ranging from 107 to 310 $\mu\text{g./100 ml.}$ of red blood cells with an average value of 170 $\mu\text{g./100 ml.}$ of red blood cells were observed. Concurrently with high concentrations of free protoporphyrin in the erythrocytes the concentrations of iron in the plasma are abnormally low.

5. Coproporphyrin was not detected in the red blood cells of either normal or vitamin B₁₂-deficient sheep. Electrophoresis on paper indicated that if coproporphyrin is present, the concentration is less than 0.2–0.3 $\mu\text{g./100 ml.}$ of red blood cells.

The author is very much indebted to her colleagues Dr L. J. Frahn, for preparing a sample of protoporphyrin IX and for help with the electrophoretic separations, and Mr B. J. Potter, for making available and assisting with the rabbits used in the experiments described. The dimethyl ester of coproporphyrin III was generously supplied by Dr M. R. Lemberg, F.R.S. The guidance of Dr E. A. Cornish in the statistical treatment of the analytical results is gratefully acknowledged.

To Mr H. R. Marston, F.R.S., Chief of the Division, the author wishes to express her sincere thanks for suggesting the problem, for making available the experimental sheep from which the blood samples were obtained, and for guidance and encouragement throughout the course of this study.

REFERENCES

- Barkan, G. & Walker, B. S. (1940). *J. biol. Chem.* **135**, 37.
 Bénard, H., Gajdos, A. & Gajdos-Török, M. (1951). *Nature, Lond.*, **167**, 990.
 Cartwright, G. E., Huguley, C. M. jun., Ashenbrucker, H., Fay, J. & Wintrobe, M. M. (1948). *Blood*, **3**, 501.
 Cartwright, G. E., Lauritsen, M. A., Jones, P. J., Merrill, I. M. & Wintrobe, M. M. (1946). *J. clin. Invest.* **25**, 65.
 Cartwright, G. E. & Wintrobe, M. M. (1948a). *J. biol. Chem.* **172**, 557.
 Cartwright, G. E. & Wintrobe, M. M. (1948b). *J. biol. Chem.* **176**, 571.
 Dresel, E. I. B. & Falk, J. E. (1956). *Biochem. J.* **63**, 388.
 Edén, A. (1940). *Nature, Lond.*, **145**, 36, 628.
 Edén, A. (1945). *Proc. Nutr. Soc.* **3**, 230.
 Fischer, H. & Pützer, H. Z. (1926). *Hoppe-Seyl. Z.* **154**, 40.
 Foster, A. B. (1952). *Chem. & Ind.* p. 1050.
 Grinstein, M. & Watson, C. J. (1943). *J. biol. Chem.* **147**, 667.
 Jope, E. M. & O'Brien, J. R. P. (1945). *Biochem. J.* **39**, 239.
 Kitzes, G., Elvehjem, C. A. & Schuette, H. A. (1944). *J. biol. Chem.* **155**, 653.
 Le Canu (1885). *C.R. Acad. Sci., Paris*, **100**, 40. [Quoted by Wade, J. (1905). *J. chem. Soc.* **87**, 1656.]
 Lemberg, R. (1954). *Fortschr. Chem. org. Naturst.* **11**, 299.
 Marston, H. R. (1952). *Physiol. Rev.* **32**, 66.
 Marston, H. R. & Lee, H. J. (1948). *J. agric. Sci.* **38**, 229.
 Marston, H. R., Lee, H. J. & McDonald, I. W. (1948a). *J. agric. Sci.* **38**, 216.
 Marston, H. R., Lee, H. J. & McDonald, I. W. (1948b). *J. agric. Sci.* **38**, 222.
 Marston, H. R. & Smith, R. M. (1952). *Nature, Lond.*, **170**, 792.
 Rimington, C. & Sveinsson, S. L. (1950). *Scand. J. clin. Lab. Invest.* **2**, 209.
 Schwartz, S. & Wikoff, H. M. (1952). *J. biol. Chem.* **194**, 563.
 Schwartz, S., Zieve, L. & Watson, C. J. (1951). *J. Lab. clin. Med.* **37**, 843.
 Watson, C. J. (1950). *Arch. intern. Med.* **86**, 797.
 Watson, C. J., Grinstein, M. & Hawkinson, V. (1944). *J. clin. Invest.* **23**, 69.

South African Pilchard Oil

5. THE ISOLATION AND STRUCTURE OF AN EICOSAPENTAENOIC ACID FROM SOUTH AFRICAN PILCHARD OIL*

By J. M. WHITCUTT† AND D. A. SUTTON

National Chemical Research Laboratory, South African Council for Scientific and Industrial Research, Pretoria, South Africa

(Received 12 December 1955)

In recent years structural diagnosis of the highly unsaturated fatty acids of natural origin has been facilitated by the increased use of spectral data, and by improvements in the methods of oxidative degradation due to Klenk & Bongard (1952). However, there is still considerable disagreement on

the suitability of some of the experimental methods that have been used in the isolation of individual acids. Thus Farmer & van den Heuvel (1938) have criticized the use of conventional vacuum-distillation techniques, and several authors have criticized the polybromide method on the grounds that structural alteration of such labile molecules can, and probably does, occur. Kolb & Brown (1955)

* Part 4: Silk & Hahn (1954b).

† Marine Oils Research Fellow.

have pointed out that the usefulness of low-temperature fractional-crystallization procedures may be limited by mutual-solubility effects.

The isolation of an eicosapentaenoic acid from South African pilchard oil (*Sardina ocellata* Jenyns) is now reported. The methods of separation used were similar to those described in earlier papers of this series (see Silk & Hahn, 1954*b*) in which the mild techniques of lithium salt-acetone segregation, urea-complex fractionation and molecular distillation were applied successively to prepare a concentrate of the required fatty acid which was then isolated by reversed-phase partition chromatography. After adapting existing oxidative and synthetic methods to small-scale use, the structure of the eicosapentaenoic acid was deduced to be the all *cis* form of *eicosapenta-5:8:11:14:17-enoic acid*.

The presence of eicosapentaenoic acids in natural products has frequently been reported in the past. Klenk (1954) refers to the presence of *eicosapenta-5:8:11:14:17-enoic acid* in fish oil, but the subsequent paper promised by Klenk & Bongard has not yet appeared. Klenk & Dreike (1955) have claimed the presence of this acid in ox-liver lipid but they did not isolate it. Toyama & Tsuchiya (1935*a*) found an eicosapentaenoic acid in sardine oil, and later (1935*b*) suggested that it was the 4:8:12:15:18-pentaene. Tutiya (1940) mentioned the presence of this acid in sardine oil, and Matsuda (1942) found it in bonito oil and supported the $\Delta^{4,8,12,15,18}$ structure with oxidative evidence. The presence of eicosapentaenoic acids has been reported by Baldwin & Parks (1943) in menhaden oil; by White & Brown (1948) in ox-adrenal lipids; by Tsuchiya & Kato (1950) in herring oil; by Shorland & Johannesson (1951) in butter fat; by Herb, Witnauer & Riemschneider (1951) in ox-adrenal lipid; by Tsuchiya & Okubo (1952) in fish-liver oil; by Toyama & Shimooka (1953), who give the alkali isomerization spectrum, in sardine oil; and by Abu-Nasr & Holman (1954), who determined some of the properties of the methyl ester, in cod-liver oil.

The data which have been given for most of the acids mentioned above do not allow any decision as to their degree of purity to be reached.

EXPERIMENTAL AND RESULTS

Melting points are uncorrected.

All unsaturated samples were stored at 4° in sealed vessels at 10⁻⁴ mm. Hg pressure; they were exposed to the atmosphere for the minimum time during manipulations and were sealed again under vacuum immediately after use. Where possible, operations were carried out under nitrogen at low temperatures. Solvents were redistilled. Ultraviolet spectra were determined on a Beckman model DU quartz spectrophotometer and infrared spectra on a Perkin-Elmer model 21 double-beam infrared spectrophotometer. For the

experiments with linoleic acid a Unicam model SP. 500 and a modified Beckman model 2T were used for the ultraviolet and infrared spectra respectively.

Isolation of the eicosapentaenoic acid

Concentration of the eicosapentaenoic acid. The starting material was the 'main-bulk residue' of Silk & Hahn (1954*b*, Table 1). Of this 38 g. was again subjected to three-stage molecular distillation to give the thirteen approximately equal fractions shown in Table 1.

Fraction 5, Table 1, absorbed 4.87 moles of hydrogen/equiv. over Pd-BaSO₄ catalyst, and on reversed-phase chromatography (Silk & Hahn, 1954*a*) most of the hydrogenation product appeared in one large peak corresponding to C₂₀ saturated acid.

Reversed-phase partition chromatography of fraction 5, Table 1. The conditions were similar to those described by Silk & Hahn (1954*b*) apart from modifications which are mentioned below.

About 0.4 g. of fraction 5 was taken up in methanol (6.5 ml.), 2 g. of kieselguhr-heptane mull was added and then water (3.5 ml.) slowly with agitation. The suspension was poured on to the top of a column (280 cm. × 1.5 cm.) of non-wetting kieselguhr supporting *n*-heptane as the stationary phase (prepared by shaking 9 g. with 8 ml.), and aqueous methanol (65%, v/v) as the mobile phase. A few millilitres of washings were added and the chromatogram was developed as described under Fig. 1, which shows the graph obtained when measured samples from the column eluate were titrated with 0.01N methanolic KOH solution. A diagram of the system used to collect and titrate the eluate was given in Silk & Hahn (1954*b*).

The acids were recovered by diluting each fraction with an equal volume of water and extracting with pentane (see Silk & Hahn, 1954*b*). In addition to the chromatogram shown in Fig. 1 three similar chromatograms were carried out later, and similar fractions, I, II and III, were isolated from each. Corresponding samples from different chromatograms were not combined with each other but were assumed to be identical for experimental purposes. Altogether, about 0.6 g. of fraction II was isolated.

Fraction I had equiv. wt. 281 and absorbed hydrogen equivalent to 4.15 double bonds/mol.

Fraction III had equiv. wt. 333 and absorbed hydrogen equivalent to 5.32 double bonds/mol.

Table 1. *Molecular distillation of the unsaturated-acid concentrate*

Fraction	Distillation temp. (°)	Equiv. wt.
Preliminary	95-105	289
1	132	287
2	132	292
3	132	298
4	148	301
5	148	299
6	148	303
7	148	305
8	148	308
9	168	316
10	168	321
11	168	333
Residue	—	—

Fig. 1. Chromatogram of fraction 5, Table 1. Weight of sample, 422 mg. Column, heptane supported on non-wetting kieselguhr, 280 cm. $\times$ 1.5 cm. Temperature, 10°. Flow rate, 100 ml./hr. Solvent system: aqueous methanol 65% (v/v) to 1500 ml. of eluate, 68% (v/v) to 1600 ml., 72% (v/v) to 1700 ml., 76% (v/v) to 1800 ml., 78% (v/v) to 3500 ml. Solvents were equilibrated with *n*-heptane. Acetic acid (0.6 ml./l.) was added to the first solvent. Eluates between arrows were collected separately as fractions I, II and III.

Properties and structure of the eicosapentaenoic acid

General properties. The eicosapentaenoic acid recovered from fraction II was a clear, faintly yellow oil of m.p. -54.4 to -53.8° , refractive index n_D^{25} 1.4977. (Found: C, 79.3, H, 10.2%. No. of double bonds/mol., 5.03 (hydrogen uptake over Pd-BaSO₄); equiv. wt., 303; iodine value, 412. C₂₀H₃₀O₂ requires C, 79.4; H, 10.0%. No. of double bonds/mol., 5.00; equiv. wt., 302; iodine value, 420.)

A Kuhn-Roth determination (Grant, 1951) gave the number of C-Me groups/mol. as 0.75.

Hydrogenation product. Chain-length analysis of the above hydrogenation product (Silk & Hahn, 1954a) showed the presence of only C₂₀ saturated acid with possible traces of C₂₂ acid.

The unsaturated acid (67 mg.) was hydrogenated over Adams's catalyst in ethanol. The product was filtered, extracted with pentane, crystallized once from pentane at -80° and dried under vacuum. It then had equiv. wt., 315, m.p., $74.8-75.0^\circ$, compared with equiv. wt., 312, m.p., 75.35° for *n*-eicosanoic acid (Francis & Piper, 1939). Mixed with synthetic *n*-eicosanoic acid (m.p., $75.0-75.3^\circ$), it had m.p., $74.8-75.1^\circ$.

The X-ray long spacing of the product was $43.7\text{ \AA}$ compared to $43.6\text{ \AA}$ for synthetic *n*-eicosanoic acid. A value of $44.15\text{ \AA}$ is given by Francis & Piper (1939) for the C modification of *n*-eicosanoic acid.

The infrared spectrum of the hydrogenation product in CS₂ was identical with that of *n*-eicosanoic acid.

Oxidation products. The unsaturated acid (80 mg.) was ozonized as described below for nonadecadienoic ester, except that no acetic anhydride was present. After decomposing with H₂O₂, KOH and HCl as below, the ether extract of the dicarboxylic acid residue proved to be completely soluble in water. Malonic and glutaric acids were identified by paper chromatography as described below. There was no trace of either oxalic acid or succinic acid.

Fig. 2. Ultraviolet spectra of fatty acids in the region 200–240 mμ. HH₁, heptadeca-2-enoic acid from Lauer, Gensler & Miller (1941). AA₁, Methyl arachidonate from Rusoff, Platt, Klevens & Burr (1945) and Barnes, Rusoff, Miller & Burr (1944). EE₁, Eicosapentaenoic acid isolated from pilchard oil.

Since no monocarboxylic acids could be isolated from the ozonolysis products, the unsaturated acid (20 mg.) was oxidized with CrO_3 under the conditions described by Eisenbraun, McElvain & Aycock (1954) and the steam distillate concentrated to small volume. Paper chromatograms showed that the only volatile oxidation products were acetic and propionic acids.

Ultraviolet absorption. Apart from an inflexion at 232 $\text{m}\mu$, corresponding to 0.5% autoxidation (Silk & Hahn, 1954b) the eicosapentaenoic acid showed no absorption in the 220–300 $\text{m}\mu$ region. The spectrum is given in Figs. 2 and 3. It can be seen from Fig. 2 that absorption in the 210–220 $\text{m}\mu$ region is closely similar to that shown by methyl arachidonate.

Alkali isomerization. The eicosapentaenoic acid (5.4 mg.) was heated at 180° under nitrogen in a 21% (w/v) solution of KOH in glycol for 8.5 min. (see Herb & Riemenschneider, 1952; Hammond & Lundberg, 1953). The absorption spectrum is shown in Fig. 3.

Infrared absorption. The spectrum shown in Fig. 4 was taken in CS_2 solution (2.06%, w/w) except from 1650 to 1320 cm^{-1} , where CCl_4 solution (1.67%, w/w) was used.

Fig. 3. Ultraviolet absorption of eicosapentaenoic acid before (broken line) and after (continuous line) alkali isomerization. For details see text.

Synthesis and degradation of heneicosapentaenoic acid. The eicosapentaenoic acid (55 mg.) was used to prepare the methyl ester (39 mg.) of its higher homologue in the same way as described below for methyl nonadecadienoate. The product was ozonized and the non-volatile fragments were chromatographed on paper. Only malonic and adipic acids were detected.

Model synthetic and degradative experiments with linoleic acid

Synthesis of methyl nonadecadi-10:13-enoate. Debromination linoleic acid (197 mg.) was mixed with oxalyl chloride (2–3 g.) at –20° in an evacuated flask. This was occasionally swirled during a period of 48 hr. at 4°. Excess of oxalyl chloride was then removed under vacuum at room temperature. The product was taken up in a few millilitres of dry ether and added slowly with swirling to a large excess of diazomethane (prepared from 0.6 g. of nitrosomethylurea) in dry ether (20 ml.) at –80°. The mixture stood under nitrogen at 0° for 2 hr. The ether and excess diazomethane were removed under vacuum and the product was taken up in dry methanol (3 ml.) and dry ether (2 ml.). To this, silver benzoate (50 mg.) dissolved in dry triethylamine (0.5 ml.) was slowly added dropwise with agitation (see Newman & Beal, 1950). The dark suspension was allowed to stand for 2 hr. under nitrogen at room temperature. Some of the solvent was then removed under vacuum and the residual solution taken up in pentane (50 ml.) and washed twice with 35 ml. of 3N-HCl, twice with 35 ml. of N-KOH and three times with 50 ml. of water. The pentane was removed under vacuum, the residue taken up in methanol (1–2 ml.) and allowed to percolate through a column of charcoal (2 cm. $\times$ 0.6 cm.) supported on a layer of non-wetting kieselguhr. Evaporation of the eluate under vacuum gave a clear colourless oil (117 mg.) which was stored in a desiccator for a few days at 0.5 mm. Hg pressure, during which time samples were being removed.

The infrared spectrum of this compound in CS_2 was almost identical with that of the methyl ester of the original linoleic acid. The extinction coefficients at 968 cm^{-1} showed that the proportion of *trans* unsaturation was the same, namely 4% of the total double bonds (Swern, Knight, Shreve & Heether, 1950). The ultraviolet spectrum was

Fig. 4. Infrared absorption of eicosapentaenoic acid. For details see text. The small band at 1520 cm^{-1} may be due to traces of CS_2 .

determined after all samples used for other purposes had been removed, and the extent of autoxidation was calculated as the molar percentage of conjugated methyl linoleate hydroperoxide in methyl linoleate, which would have the same molecular extinction coefficient (ϵ) at 232 μ . On this basis the compound showed 1.5% autoxidation compared with 0.4% in the starting material.

When the compound was catalytically hydrogenated over Pd-BaSO₄ in acetic acid it absorbed 2.01 moles of hydrogen/mol. (assuming C₂₀H₃₈O₂). After saponification the hydrogenation product had equiv. wt. 302, m.p. 67.4–67.7° compared with equiv. wt. 298, m.p. 68–65° for nonadecanoic acid (Francis & Piper, 1939).

An earlier synthesis of methyl nonadecadi-10:13-enoate is described by Karrer & Koenig (1943).

Ozonolysis of methyl nonadecadi-10:13-enoate. Some of this product (54 mg.) was taken up in dry ethyl acetate (5 ml.) and acetic anhydride (1 ml.) and cooled to –80°. A faint blue colour appeared when 3% ozonized oxygen was passed through the solution for 7 min. at 60 ml./min. A stream of nitrogen was then blown through the solution for 30 sec. Hydrogen peroxide (1 ml. of '130 vol.') and acetic anhydride (1 ml.) were added to the solution with shaking, together with a few millilitres of acetone to prevent two phases forming. The solution stood for 4 hr. at 30°, then overnight at 4°, after which it was heated in boiling water for a minute or two. The solvents were evaporated under vacuum until a colourless oil remained. This latter was heated on a water bath with 50% (w/v) KOH solution (2 ml.) for a few minutes, excess of concentrated HCl was added and the product vacuum-distilled to dryness. The distillate was collected, extracted with pentane and the latter solution washed twice with water. Evaporation of the pentane under vacuum gave a few drops of two immiscible colourless liquids (product A). The residue from the distillation was extracted several times with ether by decantation (15 ml. in all) and the extract itself evaporated to dryness and extracted twice with water (6 ml. in all). In this way the ozonolysis products were separated into fractions: A, volatile, pentane-soluble; B, 10.6 mg.; non-volatile, ether-soluble, water-soluble; C, 21.9 mg.; non-volatile, ether-soluble, water-insoluble. C was crystallized from boiling water and identified by its m.p. (131.0–131.8°) and mixed m.p. (131.0–132.6°) with a sample of sebacic acid (m.p., 131.8–132.6°).

Paper chromatography of short-chain acids. Systems (a) and (c) below are based on the methods of Lindquist & Störgards (1953) and of Isherwood & Hanes (1953). System (b) was suggested by Dr C. F. Garbers (private communication, see Cohn, Vis & Karrer, 1954).

(a) C₂–C₇ monocarboxylic acids were identified by descending development with saturated aqueous butanol for 10 hr. The acids were applied as their ethylamine salts and 0.025N aqueous ethylamine was present in the developing tank. Papers were dried in a slow current of air for 1 hr., then sprayed with 0.26% bromocresol green indicator in 50% (v/v) ethanol–butanol solution.

(b) C₂–C₈ dicarboxylic acids were developed (ascending method) for 3 hr. with a solution containing chloroform (25 ml.), formic acid (4 ml.), water (1 ml.) and acetone (9 ml.). Papers were dried as above for 4–24 hr., and sprayed with the same indicator to which just enough NaOH solution had been added to give the full alkaline colour.

(c) C₇–C₁₀ dicarboxylic acids were developed (descending method) with propanol-concentrated ammonia solution

(60:40, v/v). After drying for 1 hr. the papers were sprayed with the indicator as in (a) but spots had to be marked immediately because of fading.

The ozonolysis products A, B and C were chromatographed in these systems. A contained only caproic acid with a trace of acetic acid, B contained malonic acid, and C contained only sebacic acid. The identification of C₈ and higher acids was not possible in B because of trailing near the solvent front.

The linoleic acid used in the synthesis described above was also ozonized and caproic, malonic and azelaic acids were identified in the products.

DISCUSSION

It is impossible to state with certainty the percentage purity of the *n*-eicosapentaenoic acid obtained. Possible contaminants are closely related analogues with analytical characteristics not markedly different from those of *n*-eicosapentaenoic acid. Further, it is conceivable that a contaminant having analytical characteristics which differ in one direction may be counterbalanced by a second contaminant which differs in another. Thus the fact that the elementary analysis, equivalent weight and hydrogen uptake are within the accepted limits does not enable one to decide the purity of the specimen.

The melting-point range of the acid was 0.6°. The hydrogenated counterpart, once recrystallized from pentane at –80°, began to melt 0.5° below the upper limit of melting point of our authentic specimen of *n*-eicosanoic acid, and this point coincided with the literature value; a mixed melting point gave no depression. The X-ray long spacing gave 43.7 Å, in agreement with the result (43.6 Å) on our authentic acid; Francis & Piper (1939) recorded 44.15 Å. A reversed-phase chromatogram of the hydrogenated acid showed that octadecanoic acid was absent and that, if any docosanoic acid was present, then it was less than five per cent of the whole.

The chromatogram of the molecular distillate showed three distinct peaks of which the first and the second were well separated, whereas the third appreciably overlapped the tail of the second. In order to eliminate as much as possible of the third peak the material was chosen from the front portion of the second peak. The chromatographic curve does not enable one to assess the purity of any chosen fraction since different components may overlap so closely as to be incompletely resolved. More information as to the identity of the constituents of the other two peaks and of the way in which the acids present behave on reversed-phase columns should enable chromatographic curves to be used for the assessment of purity. Such a state of knowledge will obviously only be reached when the whole series of acids has been much more exhaustively studied.

The results relative to the purity of the *n*-eicosapentaenoic acid are probably more complete than so far exist for any other similar acid from natural sources. Nevertheless, the possibility that the *n*-eicosapentaenoic acid is a mixture of double-bond isomers still exists and is not easy to dispose of. The degradative evidence given below is consistent with the assumption that this is not the case, but small amounts of compounds other than the main one would not be detected.

The unsaturated acid contains no acetylenic linkage since its iodine value and hydrogen uptake correspond. The acid therefore has five double bonds, and these are not conjugated with each other or with the carboxyl group because the ultraviolet spectrum exhibits no bands and shows much less absorption below 240 m μ . than does *n*-heptadec-2-enoic acid.

The double bonds are all *cis* since there is no band in the infrared at 968 cm.⁻¹ (Fig. 4); there is no terminal unsaturation since no bands exist at 968 and 990 cm.⁻¹ (CH₂:CH.) or at 330 cm.⁻¹ (CH:C.) (see Bellamy, 1954). There is no distinct band at 1379 cm.⁻¹ characteristic of a CH₃ group, but this may not have appeared because of the very broad absorption which stretches from 1370 to 1500 cm.⁻¹.

Oxidation of the acid with chromium trioxide under the conditions described by Eisenbraun *et al.* (1954) yielded propionic and acetic acids as the volatile products; these were identified by paper chromatography. Presumably the acetic acid arose from further oxidation of the propionic acid. Ozonolysis yielded glutaric and malonic acids as the only identified dicarboxylic acids, whereas oxalic and succinic acids were absent. The acid could thus have any of the structures (I-V):

In choosing between these structures it is necessary to know whether the original carboxyl group appears as one of the carboxyl groups of the glutaric acid or not. The commonly used method of deciding this point is to mark the position of the original carboxyl group by esterifying it, and then to identify the half-ester formed after oxidation. However, the ease with which hydrolysis can occur during the manipulations involved led us to explore the possibility of attaching a non-removable group. Thus linoleic acid was converted by an Arndt-Eistert synthesis into methyl nonadecadi-10:13-enoate, which, on ozonolysis and hydrolysis, gave a high yield of sebacic acid together with caproic (hexanoic) and malonic acids. Repetition of the Arndt-Eistert synthesis on the *n*-eicosapentaenoic

acid, followed by ozonolysis, gave adipic and malonic acids but no succinic acid or glutaric acid; the structure is thus (I) (all *cis*-*n*-eicosapenta-5:8:11:14:17-enoic acid).

Alkali isomerization of the *n*-eicosapentaenoic acid gave ultraviolet absorption bands corresponding to conjugated pentaene, tetraene, triene and diene. This behaviour is typical of compounds containing the group

and is further confirmatory evidence for structure (I). According to Farmer (1942) double bonds separated by more than one methylene group do not move into conjugation under the usual alkali-isomerization conditions. We have checked Farmer's (1942) experiment (with squalene) and confirmed this, but it is obviously desirable that model singly and doubly methylene-interrupted polyenes not having branch methyl groups should be examined. Conjugated diene was formed in greatest amount, followed by triene, tetraene and pentaene in that order. Several authors have obtained much higher proportions of conjugated tetraenes and pentaenes (Herb *et al.* 1951; Toyama & Shimo-oka, 1953; Hammond, 1953) by alkali-isomerizing pentaene acids from other natural sources. This matter obviously needs further investigation.

SUMMARY

1. *cis*-Eicosapenta-5:8:11:14:17-enoic acid has been isolated from South African pilchard oil by the techniques of lithium salt-acetone segregation, urea-complex fractionation, molecular distillation and reversed-phase partition chromatography. Some of the properties of this acid have been determined.

2. The position of the double bond nearest to the carboxyl group has been unambiguously determined by using an Arndt-Eistert synthesis in combination with a method of ozonolytic degradation. This procedure is probably of wide applicability.

3. The degree of purity of the acid has been critically considered.

The authors wish to thank Dr W. S. Rapson for his interest throughout this work. The infrared spectra and their interpretation are due to Mr P. K. Faure and the X-ray long spacings to Mr F. R. L. Schöning. The melting point of the *n*-eicosapentaenoic acid was determined by Dr S. C. Mossop.

The investigation was made possible through the endowment of a Fellowship by Marine Oil Refiners of Africa Ltd., Simonstown, Cape Province, which is gratefully acknowledged.

This paper is published with the permission of the South African Council for Scientific and Industrial Research.

REFERENCES

- Abu-Nasr, A. M. & Holman, R. T. (1954). *J. Amer. Oil Chem. Soc.* **31**, 41.
- Baldwin, W. H. & Parks, L. E. (1943). *Oil & Soap*, **20**, 101.
- Barnes, R. H., Rusoff, I. I., Miller, E. S. & Burr, G. O. (1944). *Industr. Engng Chem. (Anal.)*, **16**, 385.
- Bellamy, L. J. (1954). *The Infrared Spectra of Complex Molecules*. London: Methuen.
- Cohn, M., Vis, E. & Karrer, P. (1954). *Helv. chim. acta*, **37**, 790.
- Eisenbraun, E. J., McElvain, S. M. & Aycock, B. J. (1954). *J. Amer. chem. Soc.* **76**, 607.
- Farmer, E. H. (1942). *Trans. Faraday Soc.* **38**, 358.
- Farmer, E. H. & van den Heuvel, F. A. (1938). *J. Soc. chem. Ind., Lond.*, **57**, 24.
- Francis, F. & Piper, S. H. (1939). *J. Amer. chem. Soc.* **61**, 577.
- Grant, J. (1951). *Quantitative Organic Microanalysis*, 5th ed. London: Churchill.
- Hammond, E. G. (1953). *Doctoral Dissertation Series*, Publication no. 7229. Ann Arbor, Michigan: University Microfilms.
- Hammond, E. G. & Lundberg, W. O. (1953). *J. Amer. Oil Chem. Soc.* **30**, 433.
- Herb, S. F. & Riemenschneider, R. W. (1952). *J. Amer. Oil Chem. Soc.* **29**, 456.
- Herb, S. F., Witnauer, L. P. & Riemenschneider, R. W. (1951). *J. Amer. Oil Chem. Soc.* **28**, 505.
- Isherwood, F. A. & Hanes, C. S. (1953). *Biochem. J.* **55**, 824.
- Karrer, P. & Koenig, H. (1943). *Helv. chim. acta*, **26**, 619.
- Klenk, E. (1954). *Naturwissenschaften*, **41**, 68.
- Klenk, E. & Bongard, W. (1952). *Hoppe-Seyl. Z.* **290**, 181.
- Klenk, E. & Dreike, A. (1955). *Hoppe-Seyl. Z.* **300**, 113.
- Kolb, D. K. & Brown, J. B. (1955). *J. Amer. Oil Chem. Soc.* **32**, 357.
- Lauer, W. M., Gensler, W. J. & Miller, E. (1941). *J. Amer. chem. Soc.* **63**, 1153.
- Lindquist, B. & Störgards, T. (1953). *Acta chem. scand.* **7**, 87.
- Matsuda, S. (1942). *J. Soc. chem. Ind. Japan*, suppl. **45**, pp. 49-51. [*Chem. Abstr.* (1951), **45**, 1511g.]
- Newman, M. S. & Beal, P. F. (1950). *J. Amer. chem. Soc.* **72**, 5163.
- Rusoff, I. I., Platt, J. R., Klevens, H. B. & Burr, G. O. (1945). *J. Amer. chem. Soc.* **67**, 875.
- Shorland, F. B. & Johannesson, D. L. (1951). *Nature, Lond.*, **168**, 75.
- Silk, M. H. & Hahn, H. H. (1954a). *Biochem. J.* **56**, 406.
- Silk, M. H. & Hahn, H. H. (1954b). *Biochem. J.* **57**, 582.
- Swern, D., Knight, H. B., Shreve, O. D. & Heether, M. R. (1950). *J. Amer. Oil Chem. Soc.* **27**, 17.
- Toyama, Y. & Shimo-oka, T. (1953). *Mem. Fac. Engng Nagoya*, **5**, 323.
- Toyama, Y. & Tsuchiya, T. (1935a). *Bull. chem. Soc. Japan*, **10**, 241. [*Chem. Abstr.* (1935), **29**, 6209^a.]
- Toyama, Y. & Tsuchiya, T. (1935b). *Bull. chem. Soc. Japan*, **10**, 301. [*Chem. Abstr.* (1935), **29**, 8378⁷.]
- Tsuchiya, T. & Kato, A. (1950). *Rep. Govt. Chem. Ind. Res. Inst., Tokyo*, **45**, 191. [*Chem. Abstr.* (1952), **46**, 2317c.]
- Tsuchiya, T. & Okubo, O. (1952). *J. chem. Soc. Japan (Ind. Chem. Sect.)*, **55**, 804. [*Chem. Abstr.* (1954), **48**, 8560e.]
- Tutiya, T. (1940). *J. chem. Soc. Japan*, **61**, 1188. [*Chem. Abstr.* (1943), **37**, 1382².]
- White, M. F. & Brown, J. B. (1948). *J. Amer. chem. Soc.* **70**, 4269.

Inhibition of the Respiration of *Trypanosoma rhodesiense* by Thiols

BY J. D. FULTON AND D. F. SPOONER

National Institute for Medical Research, Mill Hill, London, N.W. 7

(Received 3 January 1956)

During an investigation of the action of sulphydryl inhibitors and reversal agents on a normal and drug-resistant strain of *Trypanosoma rhodesiense* it was found that low concentrations of certain thiols markedly inhibited trypanosome respiration. This result was unexpected in view of the earlier work of Voegtlin, Dyer & Leonard (1923), who found that cysteine and glutathione added *in vitro* in relatively high concentrations protected trypanosomes against arsenicals without exerting a toxic effect *per se* when motility was used as a criterion. In a similar type of study Chen & Geiling (1948) found that cysteine did not affect the glycolytic rate of *T. equiperdum in vitro*, and Brand, Tobie & Mehlman (1950) did not report an inhibition of trypanosome respiration by the sulphydryl compounds which they employed as protective agents against arsenicals.

However, in contrast, Strangeways (1937) observed that the motility of *T. equiperdum* was markedly inhibited when glutathione was added to the medium of Yorke, Adams & Murgatroyd (1929) in which the trypanosomes were maintained *in vitro*. This worker found that catalase abolished the inhibition and therefore concluded that it was caused by hydrogen peroxide.

We have shown that the respiratory inhibition of *T. rhodesiense* which we have observed is caused mainly by peroxide formed during the aerobic oxidation of the thiols employed.

MATERIALS AND METHODS

Biological. The strain of *T. rhodesiense* was isolated in 1923 from a patient with sleeping sickness and has subsequently been maintained by blood passage in mice. Suspensions of trypanosomes were obtained by bleeding