it is suggested that they are the *N*-terminal substituents of at least four proteins in calf-thymus histones, namely fraction F2a and part of the groups F1, F2b and F3.

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all-*cis*-Docosa-4,7,10,13,16,19-hexaenoic Acid in Ox Retina

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The polyenoic acid present in ox retina reported by Hörhammer, Wagner & Wolff (1960) was shown by Bartley, van Heyningen, Notten & Renshaw (1962) to be a C₂₂ acid, probably with six double bonds/molecule. This finding has been confirmed and the positions of the double bonds have been determined by degradation.

MATERIALS, METHODS AND RESULTS

Isolation of methyl docosaheptaenoate. Eyes from cattle of various ages and breeds and both sexes were dissected as soon as possible after removal and the retinæ were stored at -15°. Retinæ (18 g.), 20% (w/v) potassium hydroxide solution (45 ml.) and methanol (45 ml.) were warmed at 60° for 3 hr. under nitrogen with occasional shaking. The cooled

mixture was extracted with light petroleum (b.p. 40–60°) (3 × 50 ml.) to remove unsaponified lipids and then acidified with 50 (v/v) sulphuric acid (12 ml.). The fatty acids were extracted with light petroleum (3 × 50 ml.), dried (over MgSO_4), evaporated to dryness and treated with an excess of ethereal diazomethane solution to give the methyl esters, which were recovered by evaporation.

Analysis of the methyl esters, applied by the technique of Renshaw & Biran (1962) to a Pye Argon gas chromatograph, showed the fatty acid composition to be closely similar to that reported by Bartley *et al.* (1962).

Samples of the mixed esters (50 mg.) were placed on the gas chromatograph and the methyl docosahexaenoate fractions (Found : C, 80.5; H, 9.8. Calc. for $\text{C}_{23}\text{H}_{44}\text{O}_2$: C, 80.7; H, 10.0%) were collected by the method of James (1960). The infrared-absorption spectrum of the ester, determined on the Perkin-Elmer Infracord spectrophotometer, showed peaks at 1740, 1670 and 710 cm^{-1} (liquid film).

Oxidation of methyl docosahexaenoate. (a) The ester (200 mg.), 1M-potassium carbonate (20 ml.), 0.5M-sodium periodate (100 ml.) and 0.5M-potassium permanganate (0.5 ml.) were made up to 1200 ml. with water and shaken at room temperature for 72 hr. to oxidize to carboxylic acid groups the carbon atoms joined by olefinic double bonds. The solution was washed with chloroform (200 ml.), acidified with 50% (v/v) sulphuric acid (20 ml.) and extracted with chloroform (3 × 200 ml.). The extracts were combined, washed with water (100 ml.), dried (over MgSO_4) and evaporated to dryness. The residue was warmed at 100° for 10 min. with a 10% (w/v) solution of boron trifluoride in cyclohexanol (1 ml.) to form the cyclohexyl esters (cf. Metcalfe & Schmitz, 1961). Water (20 ml.) was added after cooling and the esters were extracted with light petroleum (2 × 20 ml.). The dried (over MgSO_4) extracts were shown by gas chromatography to contain cyclohexyl propionate, dicyclohexyl malonate and dicyclohexyl succinate. (b) Methyl docosahexaenoate (50 mg.), lithium aluminium hydride (tetrahydroaluminate) (30 mg.) and anhydrous ether (10 ml.) were refluxed for 15 min. After cooling, 5% (w/v) hydrochloric acid (20 ml.) and

ether (10 ml.) were added, and the ethereal layer was separated, washed with water (10 ml.), dried (over MgSO_4) and evaporated to dryness under reduced pressure at 20° to give the docosahexaenol (41 mg.). This was dissolved in dry pyridine (2 ml.), toluene-*p*-sulphonyl chloride (50 mg.) was added and the mixture was left at room temperature for 3 hr. Then 5% (w/v) hydrochloric acid (30 ml.) and chloroform (20 ml.) were added, and the chloroform layer was separated, washed with 5% (w/v) hydrochloric acid (10 ml.), saturated sodium hydrogen carbonate solution (10 ml.) and water (10 ml.), and dried (over MgSO_4). Evaporation gave the crude docosahexaenyl toluene-*p*-sulphonate as a pale-yellow oil (42 mg.), which was reduced to the docosahexaene by refluxing with lithium aluminium hydride (50 mg.) in ether (10 ml.) for 1 hr. Acidification and extraction as before and oxidation of the crude docosahexaene under the conditions described in the previous experiment but with one-quarter of the quantities gave a mixture of mono- and di-carboxylic acids. Esterification of the oxidation products with boron trifluoride-cyclohexanol reagent and gas chromatography of the cyclohexyl esters showed the presence of cyclohexyl propionate, cyclohexyl *n*-butyrate, dicyclohexyl malonate and dicyclohexyl succinate.

DISCUSSION

The methyl docosahexaenoate was separated from the methylated fatty acids of cattle retinae by collection of samples from a gas-chromatography column. Hydrogenation has been shown to give behenic acid (Bartley *et al.* 1962) and the retention time on gas chromatography is in agreement with that for a C_{22} hexanoic acid. Analytical data confirm the formula $\text{C}_{23}\text{H}_{44}\text{O}_2$ for the methyl ester. The reaction sequences used to characterize the acid are given in Scheme 1.

Oxidation of the methyl ester (I) by the method of Lemieux & Rudloff (1955) and gas chromatography

Scheme 1

of the cyclohexyl esters of the acids produced showed cyclohexyl propionate, dicyclohexyl malonate and dicyclohexyl succinate to be present, the last two in a 5:2:1 molar ratio (calculated from the areas under the peaks on the chromatogram). Six structures are therefore possible for the docosahexaenoic acid: the double bonds could be at positions 4,7,10,13,16,19, or 3,7,10,13,16,19, or 3,6,10,13,16,19, or 3,6,9,13,16,19, or 3,6,9,12,16,19, or 3,6,9,12,15,19. Reduction of the methoxycarbonyl group of the methyl docosahexaenoate (I) to hydroxymethyl with lithium aluminium hydride to give the docosahexaenol (II), conversion of this into the toluene-*p*-sulphonyl ester (III) and further reduction with lithium aluminium hydride gave the docosahexaene (IV). Gas chromatography of the cyclohexyl esters of the oxidation products of this compound showed cyclohexyl propionate, cyclohexyl *n*-butyrate, dicyclohexyl malonate and (in small amounts) dicyclohexyl succinate to be present. The formation of *n*-butyric acid on oxidation of the docosahexaene formed by reduction of the methoxycarbonyl group of the methyl docosahexaenoate to a methyl group is possible only with the first of the six structures given above. The succinic acid present in the oxidation mixture is presumably due to the incomplete reduction of the docosahexaenyl toluene-*p*-sulphonate by lithium aluminium hy-

dride. The double bonds are therefore in the 4,7,10,13,16,19 positions in the docosahexaenoic acid. The presence of a peak at 710 cm.⁻¹ in the infrared-absorption spectrum and the absence of a peak in the 960–970 cm.⁻¹ region indicates *cis*-double bonds.

SUMMARY

1. all-*cis*-Docosa-4,7,10,13,16,19-hexaenoic acid has been isolated as the methyl ester from cattle retinae and its structure determined by degradation.

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Carbohydrates in Protein

5. PROCEDURES FOR THE ISOLATION OF GLYCOPEPTIDES FROM HEN'S-EGG ALBUMIN AND THEIR OXIDATION BY PERIODATE*

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It has been suggested that the covalent bond linking carbohydrate to protein in hen's-egg albumin is of the *N*-glycosidic type, linking the amide nitrogen of asparagine directly to the reducing carbon of a sugar molecule (Johansen, Marshall & Neuberger, 1961; Marks & Neuberger, 1961; Nuenke & Cunningham, 1961). Yamashina & Makino (1962) suggested that the linkage may be that of an imino ether, the oxygen of the amide group of asparagine being directly linked to the reducing carbon of a sugar moiety.

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To carry out further studies on the carbohydrate-protein linkage, we wished to obtain a glycopeptide containing aspartic acid as the only amino acid constituent, i.e. containing, we believe, 5 atoms of nitrogen/5 molecules of mannose (Johansen, Marshall & Neuberger, 1960, 1961; François, Marshall & Neuberger, 1962), but this has proved difficult. Most proteolytic procedures gave glycopeptides containing other amino acids in addition to asparagine or aspartic acid, and such peptides are insensitive to further attack by enzymic procedures (Jevons, 1958; Johansen, Marshall & Neuberger, 1958, 1961; Nuenke & Cunningham, 1961; Lee & Montgomery, 1962). We have there-