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ISOLATION AND CHARACTERIZATION OF 5,8,12-TRIHYDROXY-*TRANS*-9-OCTADECENOIC ACID FROM WHEAT BRAN

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Abstract—A trihydroxyacid was isolated from wheat bran under conditions commonly used for the recovery of prostaglandins from mammalian tissues. This compound was difficult to distinguish from *dinor*-$PGF_{2\alpha}$ by chromatographic methods, but was found not to be a prostaglandin. Characterization of the bran component as 5,8,12-trihydroxy-*trans*-9-octadecenoic acid was accomplished using a variety of chemical and instrumental techniques.

INTRODUCTION

IN THE course of screening plant extracts for the possible occurrence of prostaglandins, a compound (I) was detected in ethanol extracts of wheat bran that co-chromatographed with $PGF_{2\alpha}$ on Silica Gel G TLC plates in benzene–dioxane–HOAc (20:20:1)[1] and in EtOAc–Me_2 CO–HOAc (90:10:1).[2] Its methyl ester (II) co-chromatographed with CH_3-$PGF_{2\alpha}$ in $CHCl_3$–CH_3OH–H_2O(120:20:1),[3] and the trimethylsilyl (TMS) ether derivative of II eluted from a cyclo-hexanedimethanol succinate (CHDMS),[4] GLC column with *dinor*-$PGF_{2\alpha}$. However, the TMS ether of II was resolved from the corresponding derivative of *dinor*-$PGF_{2\alpha}$ by GLC on OV-1. Bran acid I showed no biological activity at concentrations up to 100 μg/ml in either the rat fundus contraction[5] or the rat colon[6] assays. A mass spectrum of acetylated II (III) was clearly incompatible with that expected for a *dinor*-PGF derivative,[7] and in fact indicated the probable absence of a ring system.

Since the natural occurrence of a compound that could be easily mistaken for a prostaglandin on chromatographic evidence alone should have significance to the many investigators working with prostaglandins as well as to those concerned with the composition of plant lipids, the characterization of the bran acid was performed as described below.

RESULTS

The i.r. spectrum of II showed hydrogen bonded-OH (3450 and 1090 cm^{-1}) and ester C=O (1740 cm^{-1}). Both II and CH_3–$PGF_{1\alpha}$ gave 0·48 as the ratio of the absorption at 3450 cm^{-1} to that at 1740 cm^{-1}, while methyl 9,10-dihydroxy stearate gave 0·33. All absorption between 4000 and 3100 cm^{-1} disappeared when II was reacted with TMS-imidazole,[8]

[1] K. GREEN and B. SAMUELSSON, *J. Lipid Res.* **5**, 117 (1964).
[2] N. H. ANDERSEN, *J. Lipid Res.* **10**, 316 (1969).
[3] G. EGLINTON, R. A. RAPHAEL, G. N. SMITH, W. J. HALL and V. R. PICKLES, *Nature* **200**, 960, 993 (1963).
[4] P. ALBRO and L. FISHBEIN, *J. Chromatog.* **44**, 443 (1969).
[5] F. COCCANI and L. S. WOLFE, *Can. J. Physiol. Pharmacol.* **44**, 933 (1966).
[6] D. REGOLI and J. R. VANE, *Brit. J. Pharmacol.* **23**, 35 (1964).
[7] M. HAMBERG, *European J. Biochem.* **6**, 135 (1968).
[8] ANON, *Handbook of Silylation*, p. 12, Pierce Chemical Co., New York (1970).

a reagent specific for -OH groups. Taken in combination with the TLC mobility, these observations support the presumption of three -OH groups in II.

An absorption peak at 970 cm^{-1} disappeared from the i.r. spectra when II or III was hydrogenated at atmospheric pressure over PtO_2. III had R_f 0·6 on TLC in $CHCl_3$–EtOAc (9:1) and this spot was absent when III was treated with dilute bromine in CCl_4 prior to TLC. A test for enols with $FeCl_3$ in $CHCl_3$ was negative with II. These experiments indicated the presence of a non-conjugated *trans* double bond.

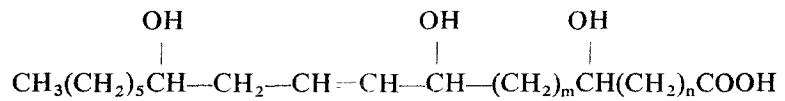
II was converted to the polymesylate[9] and reduced with $LiAlH_4$ in dry ether.[10] The product was acetylated and found to co-chromatograph (GLC) with oleyl acetate on both OV-1 and EGS. This confirmed that I contained an 18-carbon aliphatic chain and one double bond.

Under conditions giving virtually complete cleavage of methyl 9,10-dihydroxystearate,[11] periodate did not react with II. Similarly, II failed to give an isopropylidine derivative with acidic acetone, indicating the probable absence of *vicinal* hydroxyl groups.

III was cleaved at the double bond by the mild periodate-permanganate procedure of von Rudloff[12] in 40% *t*-butanol. Methyl acetyl ricinoleate was similarly cleaved as a reference. The reaction products were esterified with diazomethane and analyzed under eight sets of conditions, 150° and 200° on OV-1, CHDMS, OV-210 and EGS, liquid phases representing a wide range of selectivities for positional isomers of acetoxy-substituted fatty acid esters.[13] III gave three fragments, only one of which chromatographed with any available standard; this fragment was identified as 3-acetoxy-nonanoate by co-chromatography on all four columns.

A new compound (IV) was formed quantitatively when II was heated in aqueous acetic acid. By TLC in $CHCL_3$–CH_3OH–H_2O (120:20:1) II had R_f 0·3 and IV R_f 0·8; II showed only end absorption in the u.v. while IV had a broad absorption peak at 220–230 nm. The u.v. spectrum of IV was similar to that of 13-hydroxy-9,11-octadecadienoic acid synthesized from linoleic acid by treatment with soybean lipoxidase[14] followed by $NaBH_4$. Further evidence that IV was a conjugated diene was provided by its characteristic i.r. absorption peaks at 1600 and 1650 cm^{-1}. Compound IV differed from 13-hydroxy-9,11-octadecadenoic acid in its stability to further heating in aqueous acetic acid; the –OH group from the standard compound was apparently lost under these conditions, producing a conjugated triene (λ_{max} 257, 268, 279 nm). It thus appeared that I originally contained an hydroxyl group allylic to the double bond, but that the conjugated diene-diol presumably formed by heating in acid did not contain a —CH=CH—CH=CH—CHOH— grouping.

The data presented thus far suggest a tentative structure for I of:



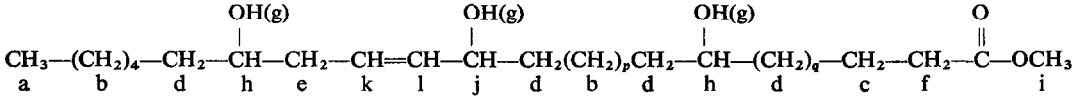
where $m \geqslant 2$ and $m + n = 5$. This partial structure was largely confirmed and expanded by NMR spectroscopy as summarized in Table 1. The absence of a triplet at 1·6 ppm (clearly

[9] W. J. BAUMANN and H. K. MANGOLD, *J. Org. Chem.* **29,** 3055 (1964)
[10] W. J. BAUMANN, L. L. JONES, B. E. BARNUM, and H. K. MANGOLD, *Chem. Phys. Lipids* **1,** 63 (1966).
[11] G. V. MARINETTI, J. ERBLAND and E. STOTZ, *J. Am. Chem. Soc* **80,** 1624 (1958).
[12] E. VON RUDLOFF, *Can. J. Chem.* **34,** 1413 (1956).
[13] A. P. TULLOCH, *J. Am. Oil Chemists' Soc.* **41,** 833 (1964.)
[14] A. L. TAPPEL, in *Methods in Enzymology* (edited by S. P. COLOWICK and N. O. KAPLAN) Vol. V, p. 539, Academic Press, New York, (1962).

TABLE 1. NMR SPECTRA OF II AND ITS DERIVATIVES*

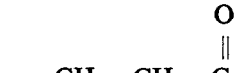
δ, ppm				
II	II-TMS	Peak type	No. of protons	Assignment
0·87	0·87	Triplet, $J = 6$	3	a
1·28	1·28			b
1·51	1·51	Not resolved	18	c
1·59	1·59			d
2·05	2·00	Multiplet	2	e
2·26	2·26	Triplet,† $J = 8$	2	f
2·87‡	—	Broad	3	g
3·45	3·40	Broad	2	h
3·63	3·63	Singlet	3	i
4·05	3·95	Tetrad, $J = 5$	1	j
5·25	5·20	Broad	1	k
5·71	5·55	Broad	1	l

* Chemical shifts relative to tetramethylsilane $\delta = 0$.
† Collapses to singlet on irradiation at 1·51 ppm.
‡ Disappears on shaking with D_2O.



Note that p or $q = 1$, the other $= 0$.

distinguishable in the spectrum of methyl 10,12-dihydroxystearate) confirmed $m \geqslant 2$. The 2·26 ppm triplet associated with a group absorbing at 1·51 ppm reflects the part structure



; hence $n \geqslant 2$ above. The hydroxyl group nearest carboxyl must therefore have occupied C-5 or C-4 of the acid I.

To distinguish between these possibilities, I was heated in toluene until the solvent had nearly all distilled. The residue, in diethyl ether, was washed with 0·1 N NaOH to remove free acids and then with water, dried over Na_2SO_4, and examined by i.r. spectroscopy. Absorption peaks at 1735 and 1250 cm^{-1} (C=O and C—O—C) were characteristic of δ- rather than γ-lactones,[15] locating the final —OH group on C-5.

The tentative structure of I was confirmed by the mass spectrum of III, scanned between *m/e* 0–700. The ions of highest mass detected were *m/e* = 439 and 411. Assuming that these correspond to M-31 and M-59, the apparent mol. wt. of 470 rules out the possibility of a ring system in addition to a carbon-carbon double bond.

Ions corresponding to M-(59 + 60), M-(73 + 60), M-(87 + 60), M-(73 + 2 × 60) and M-(31 + 3 × 60) were present, as were those corresponding to M-60, M-(2 × 60) and M-(3 × 60) but not M-(4 × 60), further confirming the mol. wt. of 470 and the presence of three acetoxy groups. A minor series of ions derived from partial cleavage of acetate units included M-(59 + 60 + 43).

Except for the usual series of hydrocarbon ions derived from the methyl end of the acid chain, the most prominent peaks in the spectrum had even *m/e* values as follows: 82 (10%),

[15] J. A. FIORITI, V. KRAMPL and R. J. SIMS, *J. Am. Oil Chemists' Soc.* **44**, 534 (1967).

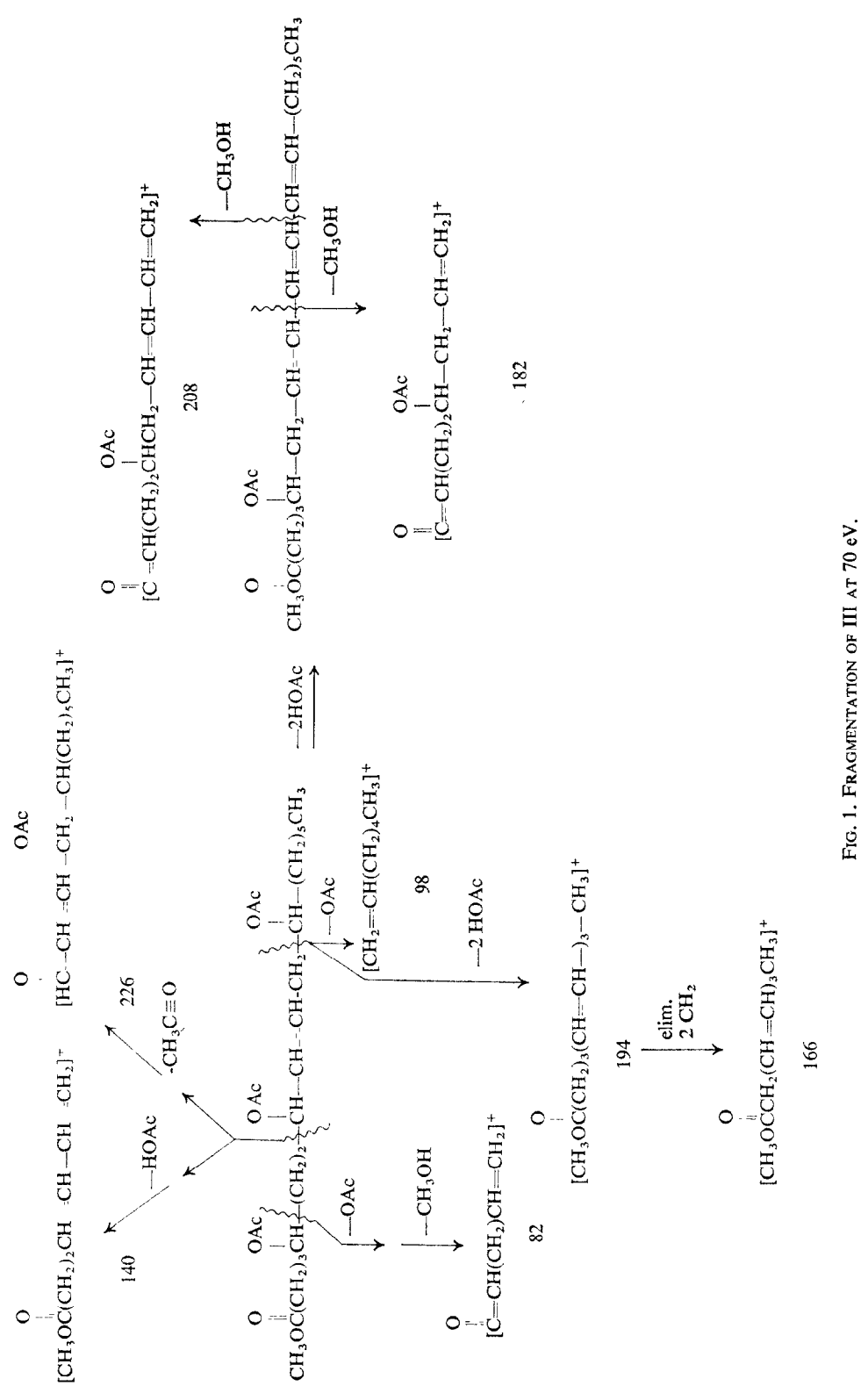


FIG. 1. FRAGMENTATION OF III AT 70 eV.

98 (9%), 140 (34%), 166 (5·4)%, 182 (9%), 194 (15%), 208 (6·6%), and 226 (24%). The most probable origins of these ions are diagrammed in Fig. 1. In each case the expected metastable ion corresponding to the final process in any $M_0^+ \rightarrow M^+$ sequence was detected within $\pm$ 0·5 *m/e* units of the calculated value.

The mass spectrum of III was thus in good agreement with the postulated structure of I as 5,8,12-trihydroxy-*trans*-9-octadecenoic acid.

DISCUSSION

Fatty acids having allylic hydroxyl groups have been reported in *Helichrysum* seed oils,[16] *Tragopogon porrifolins*[17] and *Dimorphotheca aurantiaca*,[18] generally, however, in the form of conjugated dienols. Enetriols, notably 9,10,18-trihydroxyoctadecenoate from apple[19] have been found in plants protected by cutin.

Only 50 mg of I was obtained in pure form from 5 Kg of bran in the present study, but consideration of the properties of I leaves little doubt but that significant quantities were lost as lactone and as dehydration product IV during the isolation. It is useless to speculate at the present time as to the origin, in terms of precursor, or function of I in wheat. Further studies on wheat bran acids are in progress which may suggest possible precursors of I, but tracer techniques with intact plants will be required for an understanding of the role of I in wheat metabolism.

EXPERIMENTAL

Isolation of I

5 Kg of wheat bran (Choice Quality Mills, Newberry, S. C.) was extracted in 250 g portions with 4 vol. of 95% ethanol using a Waring blender. The extracts were filtered through glass wool and then through glass fibre filter paper, after which the solvent was evaporated at 40° using a rotary evaporator. The total residue was taken up in 1 l. of $CHCl_3$–CH_3OH (2:1, v/v) and partitioned with 200 ml of 0·15 M potassium phosphate pH 9. The pH of the aqueous phase was maintained at 9 by the addition of NaOH as required.

The aqueous phase was adjusted to pH 3 with HCl and diluted with 2 vol. of water. Organic acids were extracted with 3 × 200 ml portions of Et_2O; the ether phase was washed once with water, dried and concentrated nearly to dryness at 40° *in vacuo*. The acetone- soluble portion of the residue was filtered and taken to dryness in preparation for column chromatography.

Preparation and elution of the Mallinckrodt Silic AR CC-4 column has been described previously.[4] The fraction eluted with EtOAc–Me_2CO (95:5) was taken to dryness *in vacuo* and esterified with CH_2N_2. The methyl esters at a loading of 25 mg/g absorbant were chromatographed on Adsorbosil-Cab, eluting first with Et_2O–hexane (9:1, v/v) (40 ml/g adsorbant) and then with Et_2O–Me_2CO (75:25, v/v), (40 ml/g adsorbant). The last fraction (trihydroxyesters) was concentrated and purified by preparative TLC on 250 μ plates of Silica Gel G in $CHCl_3$–CH_3OH–H_2O, (120:20:1, by vol.). The major component, as visualized on pilot strips with ethanolic phosphomolybdate, was scraped off and eluted with CH_3OH.

Spectroscopy—Spectrometry

I.r. spectra were obtained with a grating instrument from evaporated films on NaCl or AgCl plates. NMR spectra were obtained at 100 MHz in $CDCl_3$ containing 2% tetramethylsilane at 28°, using 50 μl microtubes. Mass spectra were obtained by introduction of individual samples through the solids inlet of a Perkin–Elmer Model 270 instrument operating at an ionization potential of 70 eV. U.v. spectra were scanned in *n*-hexane. The following reference compounds were used to aid the interpretation of spectra: methyl esters, acetates, and TMS-ethers of 9,10-, 9,12-, and 10,12-dihydroxystearate, 12-hydroxystearate, ricinoleate, 13-hydroxy-9,11-octadecadienoate, α-hydroxypalmitate, $PGF_{1\alpha}$, $PGF_{2\alpha}$, and $PGF_{2\beta}$.

Analytical Gas Chromatography

All analyses were made using 0·125 in. O.D. columns in a Hewlett-Packard 5750 gas chromatograph with hydrogen flame ionization detectors. The inlet and detector temperatures were 20° and 50° above the column

[16] R. G. Powell, C. R. Smith, Jr. and I. A. Wolff, *J. Am. Oil Chemists' Soc.* **42**, 165 (1965).
[17] M. J. Chisholm and C. Y. Hopkins, *Can. J. Chem.* **38**, 2500 (1960).
[18] F. R. Earle, K. L. Mikolajczak, I. A. Wolff and A. S. Barclay, *J. Am. Oil Chemists' Soc.* **41**, 345 (1964).
[19] G. Eglinton and D. H. Hunneman, *Phytochem.* **5**, 313 (1968).

temperatures, respectively. Helium was used as carrier gas at 45–50 ml/min. The column packings, lengths, and temperatures used were:

(1) *For TMS-ether methyl esters.* 3% CHDMS on 100/120 Gas Chrom Q, 2·5 m, 180° and 220°; 10% OV-1 on 80/100 HP Chromosorb W, 1·5 m, 240°.

(2) *For fatty alcohol acetates.* OV-1 as above at 200°; 12% EGS on 80/100 AW Chromosorb W, 2 m, 175°.

(3) *For permanganate/periodate cleavage products.* CHDMS, OV-1 and EGS as above at 150° and 200°; 10% OV-210 on 80/100 Supelcoport, 3 m, 150° and 200°. The equivalent chain lengths (relative to *n*-sat. fatty acid methyl esters) of methyl 3-acetoxy nonanoate were: OV-1, 12·0; CHDMS, 14·3; OV-210, 14·8; EGS, 17·4.

Chemical Methods

All chemical methods for which a reference is given were performed according to the reference without modification except that periodate oxidations[11] were run at pH 6 instead of 7·4. Dehydration with acetic acid involved 15 min at 90° in 90% acetic acid.

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