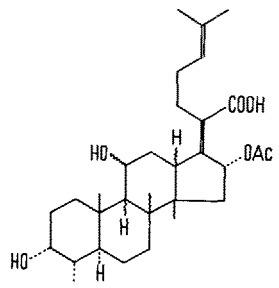
α,β-unsaturated ketone, the UV-spectrum of which indicates an extended conjugation consistent with formula XI. A compound with a similar chromophore was obtained on analogous treatment of the 16-θ-acetyl methyl ester corresponding to X.

A more direct proof for the presence of a hydroxyl group at C_{11} based upon ozonolysis of a 16-unsubstituted derivative of fusidic acid will be published in a forthcoming paper.

The arguments for the absence of a methyl group at C_9 previously set forth[1] are no longer valid in view of the new location of the hydroxyl group. However, the presence of a hydrogen atom at C_9 can now be deduced from the formation of the Δ^{9-11} compound IX as well as from the previously reported[1] experiments on the base catalyzed epimerization of derivatives of fusidic acid containing a carbonyl group in ring C. Fusidic acid is consequently believed to be represented by formula XII.



XII

Compound		M.p.	UV-spectrum (EtOH) λ max (mμ)	ε	$[\alpha]^D(CHCl_3)$
VIII	$C_{31}H_{48}O_5$	183–184°	223	14000	+ 44°
IX	$C_{31}H_{46}O_4$	143–144°	221	15500	+ 26°
X	$C_{31}H_{46}O_5$	153–154°	222	13800	+113°
XI	$C_{31}H_{44}O_5$	188–189°	280	17500	−358°

Zusammenfassung. Durch Herstellung der zwei Verbindungen IX und XI wurde erwiesen, dass die Hydroxylgruppe im Ring C der Fusidinsäure an C_{11} haften muss. Daraus sowie aus früheren Experimenten[1] lässt sich für die Fusidinsäure die revidierte Konstitutionsformel XII ableiten.

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A Group of Fatty Acids with 'Tetramethylene Interruption' in the Double Bond System[1]

Lipids from leaves and nuts of the tree *Ginkgo biloba* contain several C_{20} and C_{18} acids with double bonds in a 1,7 position relative to each other. In analogy to methylene-interrupted double bonds, they may be called 'tetramethylene-interrupted'. The group of isomers represents about 10% of the fatty acids in Ginkgo and includes nearly all the C_{20} acids. Oleic, linoleic and linolenic acids represent the greater part of the C_{18} acids; however, the isomers are present in the C_{18} series too, while they have not been found with the C_{16} chain length.

DAVIDOFF and KORN[2] found *cis,cis*-5,11-octadecadienoic acid as a major lipid constituent of the slime mold *Dictyostelium discoideum*, and BAGBY *et al.*[3] have reported *cis, cis*-5,13-docosadienoic acid from seed oil of the tree *Limmanthes douglasii*. Because several acids with such an unusual system of double bonds were detected in Ginkgo, a detailed examination of their structure and biosynthesis has been undertaken. As a first step in this direction, we report here the composition and identification of the fatty acids of *Ginkgo biloba*.

The methyl esters were fractionated by liquid–liquid chromatography[4] and gas–liquid chromatography[5,6], and the individual esters or mixtures of isomers were identified by ozonization procedures before and after alkaline isomerization. One of the structure determinations is outlined as an example. A methyl ester had been tentatively identified as an eicosatrienoate according to LLC and GLC retentions. Ozonization-reduction[7] of this ester gave a C_5 aldehyde-ester and a C_6 aldehyde which were identified by GLC. Thereby, the position of the extreme double bonds at carbons 5 and 14 were demonstrated. The fragments between these double bonds were identified by ozonization-oxidation[8]. After esterification of the resulting acids, GLC showed dimethyl adipate and glutarate in equivalent amounts. Malonate was also detected. These results locate the internal double bond at either position 8 or 11.

The exact location of this double bond can be established by alkaline isomerization and subsequent ozonization. It was shown that linoleic acid, after alkaline isomerization under standard conditions[9], yielded by ozonization-reduction C_6 and C_7 aldehydes and C_9 and C_{10} aldehyde-esters in equivalent amounts. Oleic acid remained unchanged under the isomerization conditions. Therefore, conjugation of double bonds interrupted with one methylene group proceeds equally from both sides of the unsaturated system, while an isolated double bond is

[1] This work has been supported by a research grant from the National Institutes of Health (USPHS AM-05165) and by the Hormel Foundation.

[2] F. DAVIDOFF and E. KORN, Biochem. Biophys. Res. Comm. *9*, 44, 54 (1962).

[3] M. O. BAGBY, C. R. SMITH, T. K. MIWA, R. L. LOHMAR, and I. A. WOLFF, J. Org. Chem. *26*, 1261 (1961).

[4] H. SCHLENK and J. GELLERMAN, J. Amer. Oil Chemists' Society *38*, 555 (1961).

[5] H. SCHLENK, J. GELLERMAN, and D. SAND, Anal. Chem. *34*, 1529 (1962).

[6] H. SCHLENK and D. SAND, Anal. Chem. *34*, 1676 (1962).

[7] H. SCHLENK et al., to be published.

[8] E. KLENK and W. BONGARD, Hoppe-Seyler's Z. *290*, 181 (1952).

[9] R. HOLMAN and H. HAYES, Anal. Chem. *30*, 1422 (1958).

not disturbed. This procedure was applied to the C20:3 acid of Ginkgo. It was isomerized, recovered, esterified, ozonized and hydrogenated to yield C_6 and C_7 aldehydes in equivalent amounts, but only C_5 aldehyde-ester. The structure of this acid as 5,11,14-eicosatrienoic was therewith established. The ultraviolet spectrum of the original ester showed no absorption while that after isomerization showed the normal conjugated diene peak at 233 mμ.

The infrared spectrum of the eicosatrienoate showed less than 5% *trans* double bond. This sample was recovered and hydrogenated analytically[10]. It absorbed 91% of the theoretical value for three double bonds. The hydrogenated ester was recrystallized and identified as methyl arachidate by melting point and mixed melting point.

The fatty acids of *Ginkgo biloba*

Component	% of total in		Double bond position	Relative amounts of isomers in	
	nut	leaf		nut	leaf
C14:0	2.6	1.0			
C16:0	11.3	22.3			
C16:1	6.2	3.1	7		minor
			9	major	major
			11	minor	
C16:2	tr.	tr.	7, 10	minor	major
			9, 12	major	minor
C16:3		4.8	7, 10, 13		major
			9, 12, 15		minor
C18:0	0.7				
C18:1	30.0	6.8	9	major	major
			11	minor	minor
C18:2	42.3	20.8	[a]5, 11	minor	
			9, 12	major	major
C18:3	1.6	32.0	[a]5, 11, 14	major	
			9, 12, 15	major	major
			11, 14, 17	minor	
C20:2	0.9	tr.	[a]5, 11	major	major
			11, 14	minor	minor
			14, 17		minor
C20:3	4.1	6.3	[a]5, 11, 14	major	major
C20:4	tr.	1.7	[a]5, 11, 14, 17	major	major
			9, 12, 15, 18	minor	minor

[a] Tetramethylene interrupted isomer.

Ozonization-hydrogenation, ozonization-oxidation, and ozonization-hydrogenation after isomerization elucidated the structure of the eicosatrienoic acid. These procedures were used also with the other fatty acid fractions and were given priority in that sequence when the amount of material did not suffice to carry out all the procedures mentioned above. Experience in GLC with numerous authentic isomers showed that shifts of retention time for isomers encountered in Ginkgo were consistent with the chemical evidence. The analytical results are listed in the Table.

The composition of the total fatty acids from nuts of the Ginkgo was similar to that of the leaves except for the drastic decrease of linolenic acid. This conforms with the observation that the presence of this acid is often associated with that of chlorophyll.

The occurrence of unsaturation in the C_5 position which is isolated from the other double bonds points toward biosynthetic mechanisms which so far have not been recognized. It may be noted that in spite of the presence of 5,11,14-C20:3, an acid, 5,8,11,14-C20:4 (arachidonic) is not formed by this plant.

Correlation of the unusual isomers found in Ginkgo with its early and unique place in the evolution of plant life would be most interesting. Work in that direction is underway in this laboratory[11].

Zusammenfassung. In Blättern und Nüssen von *Ginkgo biloba* wurde eine Gruppe ungesättigter Fettsäuren gefunden, die in ihrer Struktur von der üblichen Anordnung der Doppelbindungen in Fettsäuren abweichen. Jede der ungewöhnlichen Säuren hat die «isolierte» Doppelbindung in Δ^5-Position, was auf eine neuartige Biosynthese dieser Dien-, Trien- und Tetraensäuren hinweist.

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The Hormel Institute, University of Minnesota, Austin (U.S.A.), June 6, 1963.

[10] H. ROTH in *Methoden der Organischen Chemie* (Ed. E. MÜLLER, Thieme-Verlag, Stuttgart 1953) vol. II, p. 292.

[11] *Acknowledgment.* The authors wish to thank L. H. WEINSTEIN and T. H. HAINES for Ginkgo leaf extracts, T. AKIYA for nuts, and G. MIZUNO for IR-investigation of the samples.

On the Origin of the Mg^{++}-Activated ATPase of Muscle Mitochondrial Preparations

In contrast to those of the liver, muscle mitochondria have been found to exhibit a Mg^{++}-stimulated ATPase activity, even if freshly prepared and having a good phosphorylating capacity[1]. It was therefore concluded that such ATPase must either represent a normal property, specific for muscle mitochondria, or derive from some contaminating material present in the mitochondrial preparations of muscle tissue. Such contamination appears to be fairly possible, because of the existence in the muscle fibre of distinct Mg^{++}-stimulated ATPase activities associated with the myofibrils and the sarcotubular system.

In order to disclose a possible occurrence of contaminating material in muscle mitochondrial preparations, and to gain information on its origin, the effect of calcium on the Mg^{++}-stimulated ATPase activity has been investigated in pigeon breast muscle homogenate fractions obtained by differential centrifugation at various gravitational forces. In the presence of Mg^{++}, calcium does in fact activate myofibrillar ATPase while strongly inhibiting that of the sarcotubular system[2]. Further, calcium has long been known to cause structural damage in typical mitochondrial preparations, such as those of liver, resulting in a substantial increase of the Mg^{++}-stimulated ATPase.

Material and Methods. Mitochondria were prepared from pigeon breast muscle. The tissue was minced and homogenized in 6 vol of *M* sucrose with a lucite Potter

[1] G. F. AZZONE, E. CARAFOLI, and U. MUSCATELLO, Exp. Cell Res. *21*, 456 (1960).

[2] U. MUSCATELLO, E. ANDERSSON-CEDERGREN, G. F. AZZONE, and A. VON DER DECKEN, J. Biophys. Biochem. Cyt. *10*, Suppl. 4, 201 (1961).