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STUDIES ON THE CONSTITUENTS OF UMBELLIFERAE PLANTS—VII

STRUCTURE OF LIGUSTILIDE 2

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Abstract—A revised structure (Id) for ligustilide, a new phthalide from Umbelliferae plants, has been proposed and the structures of related substances elucidated. Biogenetic conclusions are drawn by making comparison of the structure with that of other phthalides from the Umbelliferae.

PREVIOUS papers report the isolation of ligustilide and a preliminary structural investigation.[1,2]

Ligustilide was first isolated from the roots of Hokkai-Toki, a variety of *Ligusticum acutilobum* Sieb. et Zucc. but lately it has been found that the roots of Senkyu, *Cnidium officinale* Makino (Umbelliferae), are a much better source of ligustilide and the recent investigations were made on the material from Senkyu.

The constituents of Toki and Senkyu include a variety of compounds containing the 3-butyl phthalide skeleton.[3–5] Other plants belonging to the Umbelliferae, such as celery (*Apium graveolens L.*)[6] and livèche (*Levisticum officinale* Koch.)[7] also contain compounds of this type.

Catalytic hydrogenation of ligustilide affords dihydro-(II), tetrahydroligustilide (III), and 3-butyl phthalide (IV). Sedanonic acid (V) is obtained by alkaline hydrolysis of II and similarly 2-pentanoylcyclohexanecarboxylic acid (VI) is derived from III. On the basis of these results, three possible structures (Ia, Ib and Ic) were tentatively postulated for ligustilide,[2] but after further investigation, a new structure (Id) is now proposed.

Partial hydrogenation of I affords small amounts of III and IV together with a better yield of dihydroligustilide (II). The latter (II) has a strong band at 273 mμ (log ε 4·25) in the U.V. spectrum and affords adipic and butyric acid on ozonolysis. Therefore, structure II was proposed although this is not readily reconciled with the fact that sedanonic acid (V) is obtained by hydrolysis of II. Similar examples, however, are found in the isomerization of Δ^1 to Δ^2-tetrahydrophthalic acids and in citraconic

[1] H. Mitsuhashi, U. Nagai, T. Muramatsu and H. Tashiro, *Chem. & Pharm. Bull.* **8**, 243 (1960).
[2] H. Mitsuhashi, U. Nagai and T. Muramatsu. *Chem. & Pharm. Bull.* **9**, 115 (1961).
[3] Y. Murayama, *J. Pharm. Soc. Japan* **No. 477**, 951 (1921); Y. Murayama and T. Itagaki, *ibid.* **No. 493**, 143 (1923).
[4] T. Noguchi, *J. Pharm. Soc. Japan* **54**, 913 (1934); T. Noguchi, S. Fujita and K. Kawanami, *Ibid.* **57**, 761 (1937); T. Noguchi and M. Kawanami, *Ibid.* **57**, 778, 783 (1937).
[5] T. Kariyone and M. Kanno, *J. Pharm. Soc. Japan* **56**, 662 (1936); T. Kariyone and R. Sugino, *Ibid.* **56**, 668 (1936); T. Kariyone and M. Kotani, *Ibid.* **57**, 799 (1937).
[6] G. Ciamician and P. Silber, *Ber. Dtsch. Chem. Ges.* **30**, 492, 501, 1419, 1424 and 1427 (1897); D. H. R. Barton and J. X. deVries, private communication.
[7] Y. R. Naves, *Helv. Chim. Acta* **26**, 1281 (1943).

to itaconic acids.[8] Dihydroligustilide (II) has been isolated *in situ* from Toki and it is considered that sedanonic anhydride, which is present in other Umbelliferae plants[4,6,7] but has not been isolated in a pure state, is identical with (II). Naves[7] and Noguchi *et al.*[4] assigned the structure VIIa to the phthalazone obtained by the action of hydrazine hydrate on sedanonic acid but on the basis of the U.V. spectrum (λ_{max} 282 mμ, ε 3770) and a comparison with that of 3-pyridazone (λ_{max} 280 mμ, ε 3550)[9] it is proposed that VII is the correct structure. (Chart 1).

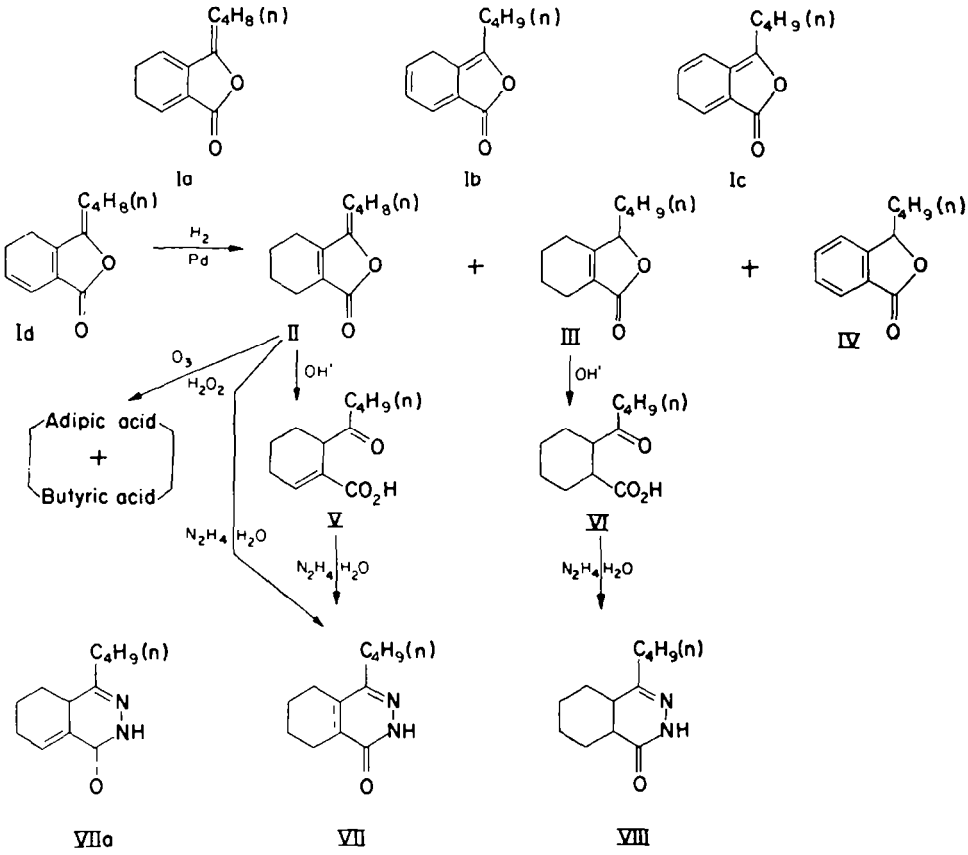


CHART 1

Tetrahydroligustilide (III) has a band at 217 mμ (log ε 4·08) in the ultraviolet and yields a keto-acid on alkaline hydrolysis. These results require the presence of a $\Delta^{\alpha,\beta}$-butenolide moiety as in the structure III. A similar example is found in the hydrolysis of β-cyclohexyl-$\Delta^{\alpha,\beta}$-butenolide,[10] studied as a model compound for cardic steroids.

The nuclear magnetic resonance spectrum of ligustilide indicates the presence of a butyliden-side chain and three olefinic protons. These requirements allow structures Ia, Id, Ie, and If but only Id and Ie explain the strong U.V. absorption at 320 mμ and

[8] A. Bayer, *Liebigs Ann.* **258**, 165 (1890).
[9] W. G. Overend, J. M. Turton and L. F. Wiggins, *J. Chem. Soc.* 3500 (1950).
[10] W. D. Paist, E. R. Blout, F. C. Uhle, and R. C. Elderfield, *J. Org. Chem.* **6**, 273 (1941).

also the unsymmetrical peaks at low field in the nuclear magnetic resonance spectrum. (Fig. 1 and Chart 2).

A Diels-Alder reaction of ligustilide with maleic anhydride yields an adduct $C_{16}H_{16}O_5$(IX) which has an ultraviolet absorption at 221 mμ (log ε 3·93) and yields three isomeric acids (X) on alkaline hydrolysis. These acids are all tri-basic, all have a strong absorption at 214 mμ in the ultraviolet and the I.R. spectra of their tri-potassium salts, all have a common carbonyl band at 1685 cm^{-1}. This behaviour of the adduct (IX) suggests the presence of a $\Delta^{\alpha,\beta}$-unsaturated enol-lactone, which

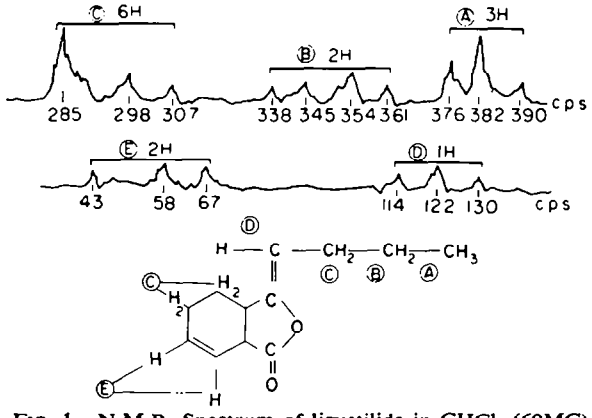
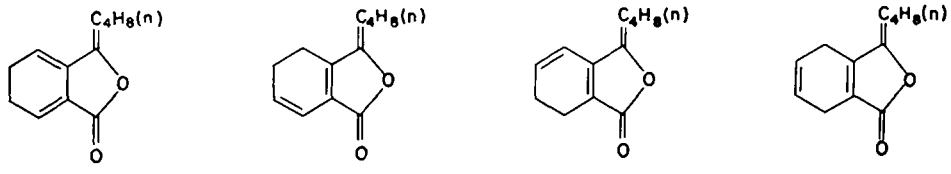


FIG. 1. N.M.R. Spectrum of ligustilide in $CHCl_3$ (60MC)



Ia Id Ie If

CHART 2

hydrolyses to the corresponding keto-acid (X). These acids are tri-basic with two additional carboxyl groups from the maleic residue, and are considered to be diastereoisomeric with respect to the configuration of the carboxyl group, which can be epimerized under the condition of alkaline hydrolysis, and/or tautomeric concerning the conversion of keto-acid to lactonol. Chart 4 shows the possible structures of the keto acids. The other carboxyl groups are unlikely to fuse lactone rings because of their high strain. The structures shown in Chart 4 do not imply the absolute configuration. (Charts 3 and 4).

Possible structures of the adduct (IX) and the corresponding keto-acids (X), derived from the proposed structures (Id and Ie) for ligustilide are shown in Chart 3. The structure (IXa), which contains $\Delta^{\alpha,\beta}$-unsaturated lactone, is considered the correct structure for the adduct.

The ozonolysis of the keto-acid (X) decomposing at 183°, confirms this structure assignment. Hydrogen peroxide oxidation of the ozonide under slightly alkaline condition affords an acid (XI) m.p. 185–187° and the molecular formula $C_{14}H_{20}O_7$, which is in agreement with the structure XIa derived from Xa via the route shown

in Chart 5. On the other hand, ozonolysis of the keto-acid (Xb) under the similar conditions should yield the acid (XIb), the elemental composition of which is quite different from the observed value. Thus, the structure Xa and also the structures, IXa and Id are considered correct.

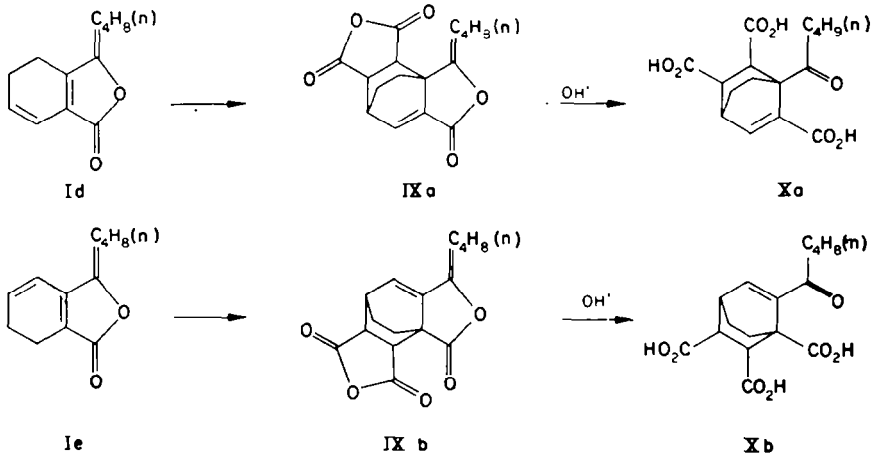


CHART 3

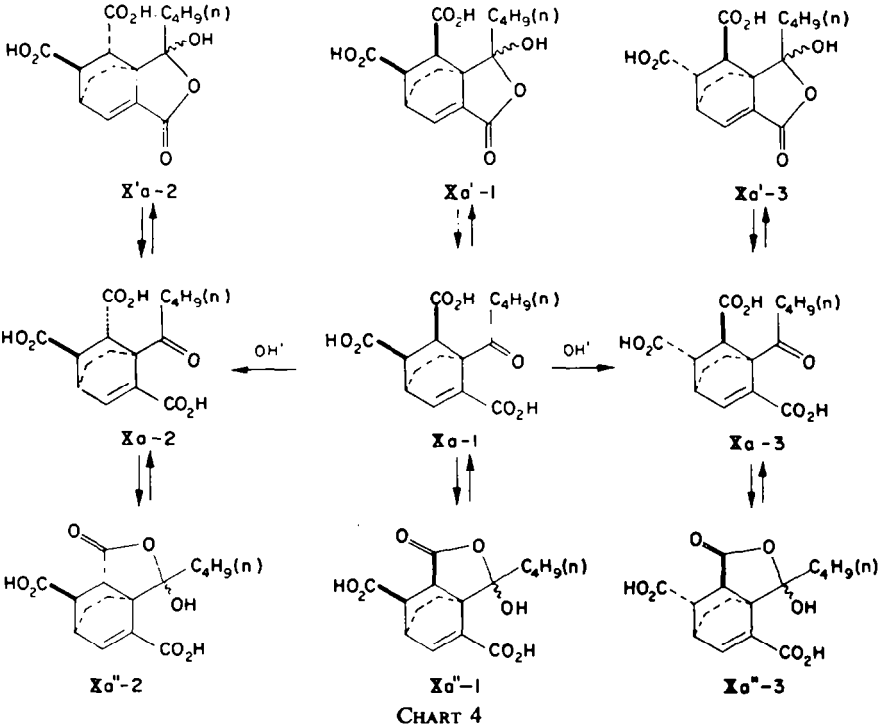


CHART 4

However, the problem of double bond migration during the reactions requires elucidation. Double bond migrations are unlikely during the hydrolysis of the adduct (IX) and ozonolysis of the keto-acid (X), since the double bond concerned is in a

(2.2.2)-bicycloöcten skeleton and Bredt's rule forbids a bridge-head double bond in such bicyclic systems.

Migration during the Diels-Alder reaction also seems unlikely if one considers that ligustilide was distilled at about 150° *in vacuo* during purification and the structure (Id) can explain the spectral properties. The authors also would expect

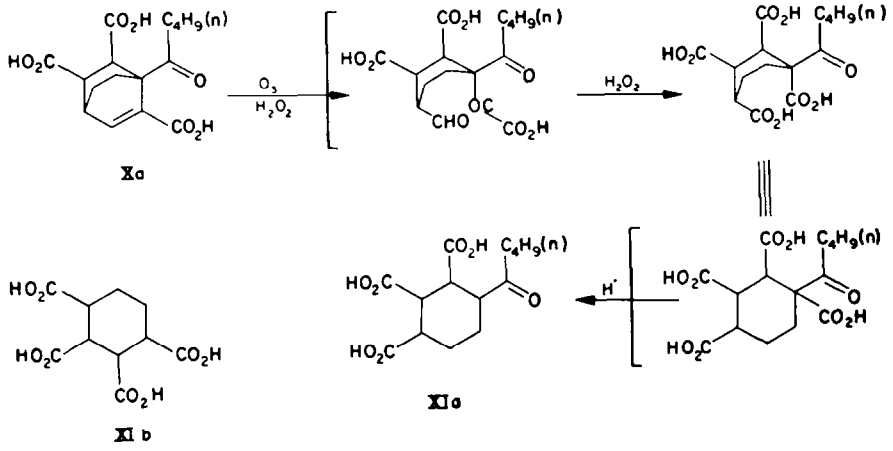


CHART 5

the other possible cyclohexadiene systems for ligustilide to react with maleic anhydride *in situ*.

Recently, the structure of cnidium lactone (XII) from Senkyu was established in this laboratory.[11] Biogenetically it is interesting to note that the two lactones isolated from the same plant, Senkyu, each have a double bond at Δ^6 on the phthalide skeleton, and that the other two double bonds in ligustilide are located at the same positions as the double bonds in the structure (II) proposed for sedanonic anhydride present

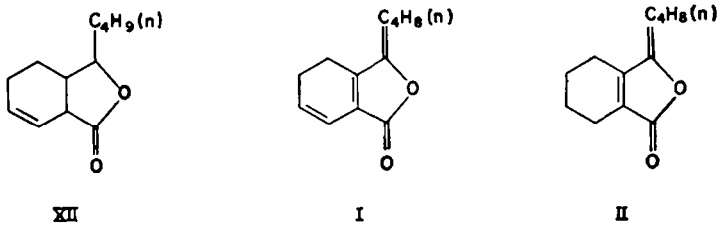


CHART 6

in other plants belonging to the Umbelliferae. These facts substantiate the ingeneous suggestion by Professor D. H. R. Barton and Juan X. de Vries[6] that the biogenesis of these phthalides may involve the head-to-tail linkage of six "acetate" or "malonate" units. Experiments are in progress to prove this theory by the use of labelled acetate. (Chart 6).

EXPERIMENTAL

M.p.s are uncorrected. U.V. spectra are for ethanol solutions unless otherwise stated. Silicic acid of Mallinkrodt 100 mesh for chromatography was used in all cases.

[11] H. Mitsuhashi, U. Nagai, T. Muramatsu and M. Tani, *5th Symposium on the Organic Chemistry of Natural Products*, Sendai, Japan (1961).

Preparation of dihydrolingustilide (II). Lingustilide (6·8 g) in hexane (45 ml) was shaken in hydrogen at 15° and atm. press. with 5% palladized barium carbonate (0·7 g) until 410 ml hydrogen was absorbed. The catalyst was filtered off and the solvent removed *in vacuo*. The residual oil (6·0 g) was chromatographed on a silicic acid column (200 g) with chloroform as an eluting solvent. The fractions with strong U.V. absorption at 276 mμ in eluted chloroform solution itself, were collected. After removal of the solvent *in vacuo*, crude dihydroligustilide (3·1 g) was obtained and chromatographed again before use. λ_{max} 272 mμ (log ε 4·25) ν_{max}^{liq} 1755 (γ-lactone), 1685 and 1647 (conjugated C=C) cm^{-1}. (Found: C, 75·77; H, 8·24; $C_{12}H_{16}O_2$ requires: C, 74·97; H, 8·39%).

Ozonolysis of dihydroligustilide (II). Ozonized oxygen (3%) was passed through a solution of dihydroligustilide (2.3 g) in chloroform (50 ml) until the ozone-consumption became constant (2 hr). After removal of the solvent *in vacuo* the residue was treated with a solution of sodium hydroxide (1·5 g) and 30% hydrogen peroxide (3 ml) in water (20 ml), and stirred 1 hr in an ice-bath. The same amount of the alkaline hydrogen peroxide solution was added again and the reaction completed by warming at 60° for 1 hr and then left overnight at room temp. After acidification, the solution was steam distilled. The distillate (100 ml) was titrated with 1N NaOH (f = 0·9548), 6.25 ml of which was consumed. Yield 50%.

The neutral solution was evaporated to dryness, and the residue (0·8 g) was partly subjected to paper chromatographic analysis[12] (Toyo-roshi No. 50 as paper; n-BuOH saturated with 1·5N NH_4OH as solvent).

The remaining portion of the residue was acidified, extracted with ether continuously, and the ethereal solution concentrated and subjected to gaschromatographic analysis.[13] [Shimadzu GC-2A; column (length 3 m, diameter 0·4 cm); stationary phase, SiDC 550-Stearic acid; temp. 113–114°; carrier gas, hydrogen (rate, 60 ml/min)]. Butyric acid was identified by both analyses, and a trace of formic acid also found.

The residue from the steam-distillation was extracted continuously with ether and the non-volatile acid fraction (1·9 g) obtained upon evaporation of the solvent. Recrystallization from ether gave an acid (0·7 g) m.p. 150–151°, which showed no depression on admixture with authentic adipic acid. From the mother liquors more adipic acid (0·5 g) of somewhat lower m.p. was obtained. In addition to a large spot of adipic acid, traces of glutaric and succinic acid were identified by paper-chromatography of the non-volatile acid fraction [Toyo-roshi: No. 50 as paper; EtOH:28% NH_3: H_2O (16:1:3) as solvent]. Trace acids were considered to be formed by abnormal degradation of the ozonide[14] and/or from some impurities in dihydroligustilide.

Hydrolysis of dihydroligustilide (II). Dihydroligustilide (30·7 g) was refluxed 1 hr with 5% ethanolic potassium hydroxide (500 ml). Water (500 ml) was added and the solution concentrated *in vacuo* until the distillate was 500 ml. The resulting aqueous alkaline solution was washed with ether, saturated with carbon dioxide, washed again with ether, acidified and extracted with ether. The acid fraction (24·9 g) obtained from the ethereal solution was crystallized from ethyl acetate–hexane (1:10).

The crude crystals (9·2 g) were recrystallized from ethyl acetate–hexane (1:5). Sedanonic acids of m.p. 108–110° (5·4 g) and of m.p. 105–108° (1·1 g) were obtained. Yield 19·3%.

Preparation of tetrahydroligustilide (III). Ligustilide (490 mg) in hexane was shaken in hydrogen at 20° atm. press. with 5% palladized charcoal (100 mg) until 104 ml hydrogen was absorbed. After filtering off the catalyst, the solvent was removed *in vacuo*. The residual oil was chromatographed on a silicic acid column (10 g) with chloroform as the eluting solvent. Each fraction was evaporated *in vacuo* and subjected to I.R. measurement. The fractions of characteristic I.R. pattern, were collected (290 mg). λ_{max} 217 mμ (log ε 4·08). $\nu_{max}^{CHCl_3}$ 1740 (α,β-unsaturated γ-lactone), 1675 (conjugated C=C) cm^{-1}. (Found: C, 73·59; H, 9·30. $C_{12}H_{18}O_2$ requires: C, 74·19; H, 9·34%).

Diels-Alder reaction of ligustilide with maleic anhydride. A warm solution of maleic anhydride (15.0 g) in xylene (100 ml) was poured into a thick-walled glass-tube containing ligustilide (3.0 g). The tube was saturated with nitrogen, sealed, and heated in an oil-bath of 130–150° for 28 hr. Xylene and excess of maleic anhydride were removed by distillation under red. press. Recrystallization of

[12] F. Brown and L. P. Hall, *Nature, Lond.* **166**, 66 (1950).

[13] A. T. James and J. P. Martin, *Biochem. J.* **50**, 679 (1952).

[14] R. H. Eastman and R. M. Silverstein, *J. Amer. Chem. Soc.* **75**, 1493 (1953); W. G. Young, A. C. McKinnis and J. D. Roberts, *Ibid.* **68**, 993 (1946).

the residue twice from benzene yielded a crystalline adduct (IX) (0·7 g); m.p. 164–166°. λ_{max} 221 mμ (log ε 3·93). ν_{max}^{nujol} 1860, 1840–1825 (doublet), 1770 (anhydride and γ-lactone), 1705 (enolic C=C), 1645 (conjugated C=C) cm^{-1}. (Found: C, 66·62; H, 5·38. $C_{16}H_{16}O_8$ requires: C, 66·66; H, 5·59%).

Hydrolysis of the adduct (IX). The adduct (0·8 g) was refluxed 2 hr with 5% ethanolic potassium hydroxide (30 ml). Ethanol was replaced with water by adding water dropwise at the same rate ethanol distilled off under red. press.

The resulting aqueous alkaline solution was washed with ether, acidified and extracted continuously with ether for 16 hr. During extraction, crystals separated out. The crystals were treated with hot ethyl acetate and separated into two fractions, soluble and insoluble. The soluble fraction was recrystallized from ethyl acetate yielding A (0·3 g) m.p. 180–182° (dec.) λ_{max} 214 mμ (log ε 3·82) ν_{max}^{nujol} 3620–3550 (doublet) (lactonel OH), 1780 (lactonol C=O), 1710–1690 (broad) (ketone and carboxyls), 1618 (conjugated C=C) cm^{-1}. (Found; C, 58·94; H, 6.42. $C_{16}H_{20}O_7$ requires: C, 59·25; H, 6·22%). The insoluble fraction was recrystallized from acetone yielding B (0·1 g) m.p. 162–164° (dec.). λ_{max} 211 mμ (log ε 3·79). ν_{max}^{nujol} 3560 (crystalline H_2O), 1710–1690 (broad) (ketone and carboxyls), 1615 (conjugated C=C) cm^{-1}. (Found: C, 55·60; H, 6·08. $C_{16}H_{20}O_7 \cdot H_2O$ requires; C, 56·13; H, 6·48%). The ethereal residue (0·6 g) was recrystallized from benzene yielding C (0·1 g) m.p. 230° (dec.). λ_{max} 214 mμ (log ε 3·91). ν_{max}^{nujol} 3500–3350 (doublet) (crystalline H_2O), 1728 (ketone), 1705 (carboxyl), 1683 (α,β-unsaturated carboxyl), 1618 (conjugated C=C) cm^{-1}. (Found: C, 55·97; H. 6·37. $C_{16}H_{20}O_7 \cdot H_2O$ requires: C, 56·13; H, 6·48%), after drying at 100° 3 hr, over phosphorous pentoxide. (Found: C, 59·23; H, 6·17. $C_{16}H_{20}O_7$ requires: C, 59·25; H, 6·22%).

Tri-potassium salts of adduct-hydrolyzates (X). The keto-acids (crystals A, B, and C) were dissolved in small amounts of water. Three equivalents of potassium carbonate was added to the solution. The neutral solution was evaporated *in vacuo* and dried in an evacuated desiccator over calcium chloride. The resultant salts were all soap-like and considered to contain some water. They were subjected to I.R. measurements in KBr without further drying.

Ozonolysis of adduct-hydrolyzate (X). Ozonized oxygen (3·5%) was passed through a solution of keto-tricarboxylic acid (130 mg) m.p. 180–182° (dec.) in ethyl acetate (15 ml) for 1·5 hr. The solution was extracted with 5% sodium bicarbonate containing 10% hydrogen peroxide. The alkaline hydrogen peroxide solution was kept 1 hr at room temp, and then carefully heated 15 min on a boiling water-bath.

The solution was acidified, left overnight and treated with sodium hydrosulphite solution dropwise until a negative peroxide test with potassium iodide was observed. The white turbidity of sulphur formed, was removed by centrifugation and the supernatant extracted continuously with ether. The ethereal residue (85 mg) was subjected to partition chromatography.[15] The column was prepared with silicic acid (20 g), water (12 ml) and chloroform (80 ml). The sample was dissolved in small volume of chloroform–butanol mixture, charged into the column and eluted with chloroform–butanol mixture (100 ml for each composition) in the following sequence. [Chloroform–butanol (100:0), (95:5), (90:10), (85:15), (80:20), (75:25), (70:30), (60:40), and (50:50)]. Fractions were collected by 10 ml for each. The three fractions, No. 21–23, were combined evaporated and the residue (19 mg) was recrystallized thrice from ethyl acetate yielding (3 mg) m.p. 185–187°. (Kofler-block). ν_{max}^{nujol} 1720–1700 (broad) (ketone and carboxyls) cm^{-1}. (Found: C, 55·94; H, 6·56. $C_{14}H_{20}O_7$ requires: C, 55·99; H, 6·71%).

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* Since we had almost finished our research, we received communication from Professor Barton that one of his graduate students (Mr. Juan X. deVries) is also doing a similar study on celery and sent us their report. Their suggestion on biogenesis seems to support the structures here proposed.

[15] C. S. Marvel and R. D. Rands, Jr., *J. Amer. Chem. Soc.* **72**, 2642 (1950).