815. *Some Degradation Products of Fumagillin.*

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Fumagillin is a half ester of octatetraene-1 : 8-dicarboxylic acid with an alcohol, $C_{16}H_{26}O_4$, which contains an epoxide ring and two other rings, one of which contains oxygen. One carbon atom of the epoxide ring carries a 3-methylbut-2-enyl group while the other is probably part of a *cyclo*hexane ring. Ozonolysis effects the step-wise removal of these groups and subsequent reduction yields alcohols containing ten and eleven carbon atoms. The ring system in these alcohols has not been identified, but their behaviour with oxidising agents excludes some possible structures.

THE discovery of fumagillin followed observations of its activity against bacteriophage.[1,2] Fumagillin has had only moderate success as a drug for the treatment of amœbiasis, but its outstanding activity against *Entamoeba histolytica in vitro*, and its other biological properties make the elucidation of its structure a matter of particular interest. It has been shown[3,4] to be a half ester of octa-1 : 3 : 5 : 7-tetraene-1 : 8-dicarboxylic acid, but further progress has been hindered by the instability of fumagillin [5] and its derivatives, and by the considerable difficulty of obtaining crystalline, or even homogeneous, degradation products.[6,7]

Fumagillin is rapidly hydrolysed by cold 0·1N-sodium hydroxide solution, giving octa-1 : 3 : 5 : 7-tetraene-1 : 8-dicarboxylic acid and an alcohol, $C_{16}H_{26}O_4$, for which the name "fumagillol" is proposed. This alcohol was first obtained as a syrup which crystallised only slowly after purification by counter-current distribution between *n*-hexane and water (the observed distribution was close to that calculated for a pure substance), but later preparations crystallised without difficulty. The infrared spectra of syrupy, crystalline, and distilled samples were identical. Schenck [6] and Tarbell [7] and their collaborators employed more drastic conditions of hydrolysis and obtained only a syrup which they called "alcohol I." Fumagillol contains one methoxyl group, two *C*-methyl

[1] Eble and Hanson, *Antibiotics and Chemotherapy*, 1951, **1**, 54.
[2] Asheshov, Strelitz, and Hall, *ibid.*, 1952, **2**, 361.
[3] Schenck, Hargie, Tarbell, and Hoffman, *J. Amer. Chem. Soc.*, 1953, **75**, 2274.
[4] Brown and Landquist, *Chem. and Ind.*, 1953, 973.
[5] Garrett, and Eble, *J. Amer. Pharm. Assoc.*, 1954, **43**, 385; Eble and Garrett, *ibid.*, p. 536; Garrett, *ibid.*, p. 539.
[6] Schenck, Hargie, and Isarasena, *J. Amer. Chem. Soc.*, 1955, **77**, 5606.
[7] Tarbell, Hoffman, Al-Kazimi, Page, Ross, Vogt, and Wargotz, *ibid.*, p. 5610.

groups, one active hydrogen atom, and no carbonyl group. Hydrogenation indicates that there is one double bond; infrared data (bands at 830 and 1673 cm.$^{-1}$ absent in dihydrofumagillol), and the ultraviolet absorption spectrum of the tetranitromethane adduct [8] show this to be doubly or triply substituted.

The nature of the hydroxyl group is uncertain, but the ease of hydrolysis of fumagillin and the failure of fumagillol to give characterisable esters or similar functional derivatives suggest that it is tertiary. The infrared spectrum shows strong absorption at 1095 cm.$^{-1}$ which might be attributable to an acyclic secondary alcohol, to a cyclic tertiary alcohol, or to the methoxyl group. The hydroxyl stretching frequency (3571 cm.$^{-1}$) in dilute carbon tetrachloride solution suggests that intramolecular hydrogen bonding occurs. An attempted Oppenauer oxidation gave a syrup which showed only faint carbonyl absorption; a secondary alcohol should have given a more definite result. Tarbell *et al.*[7] obtained a ketone, $C_{16}H_{24}O_4$, by oxidation of " alcohol I " with chromium trioxide in pyridine, but their product contained a hydroxyl group. The formation of this must have involved fission of a cyclic ether link, and the carbonyl group might have originated from oxidation at this point and not from the original hydroxyl group.

Fumagillol reacts rapidly with cold dilute (0·1N) hydrochloric acid, giving a chlorohydrin, probably $C_{16}H_{27}O_4Cl$, containing two active hydrogen atoms. It rapidly liberates one equivalent of alkali from sodium thiosulphate solution, giving a product which on alkaline hydrolysis yields a disulphide, $C_{32}H_{54}O_8S_2$. These reactions suggest the presence of an epoxide ring, since cyclic ethers with larger rings react slowly or not at all, and addition of hydrogen chloride at the double bond would not produce an additional active hydrogen atom. The reaction with thiosulphate is also given by dihydrofumagillol. The formation of a carbonyl compound (with loss of reactivity to thiosulphate) when fumagillol is boiled with magnesium bromide in butyl ether, and the loss of reactivity to thiosulphate on reduction with lithium aluminium hydride or on acid or alkaline hydrolysis, are further indications that an epoxide group is present. Alkaline hydrolysis of fumagillin in methanol affords a syrupy alcohol with twice the methoxyl content of fumagillol, presumably through addition of methanol at the epoxide group. Tarbell *et al.*[7] found that hydration occurs on treatment of " alcohol I " with aqueous sodium hydroxide, and hydration or addition of ethanol during the acid hydrolysis of fumagillin. The compound $C_{23}H_{31}O_9N_2Cl$ obtained from dihydrofumagillol and 3 : 5-dinitrobenzoyl chloride [6] is presumably formed by the addition of the acid chloride or hydrogen chloride to the epoxide group, but the presence of two active hydrogen atoms in this compound is not readily explained. It has not been possible to demonstrate a reaction between sodium thiosulphate and fumagillin or its methyl ester, possibly because of interference by the carboxyl or ester group, but it is unlikely that the epoxide group is formed during the hydrolysis of fumagillin, particularly as this would require a free hydroxyl group, *i.e.*, a second active hydrogen atom, in the fumagillin molecule. It is significant that a strain of *Aspergillus fumigatus* other than the ones producing fumagillin has been shown to produce epoxysuccinic acid.[9]

Hydrolysis of the epoxide ring in fumagillol proceeds slowly in alkaline solution, but more rapidly in dilute sulphuric acid, giving syrups with complex infrared spectra devoid of carbonyl bands. The product obtained with cold dilute sulphuric acid appeared to be the most homogeneous, and on oxidation with periodate it consumed 0·75—1·5 mols. in neutral solution and 1·5—2·3 mols. in acid solution. Among the oxidation products were acetone and a volatile C_6 carbonyl compound which were isolated as their 2 : 4-dinitrophenylhydrazones. The non-volatile oxidation product was an intractable syrup which decomposed on attempted distillation in a high vacuum. From the behaviour of fumagillol on ozonolysis, it appears likely that the C_6 carbonyl compound is 4-methylpent-3-enal, but attempts to confirm this by synthesis of the aldehyde from 4-methylpent-3-enoic acid were unsuccessful: distillation of the calcium salt with calcium formate gave only small amounts of carbonyl compounds, of which only acetaldehyde was identified, and the main product appeared to be a lactone (infrared absorption at 1778 and 1763 cm.$^{-1}$); reduction of the *N*-methylanilide with lithium aluminium hydride gave a complex mixture; and treatment

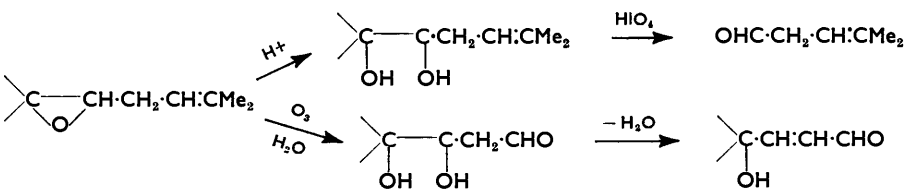
[8] Heilbronner, *Helv. Chim. Acta*, 1953, **36**, 1121.
[9] Birkinshaw, Bracken, and Raistrick, *Biochem. J.*, 1945, **39**, 70.

of the ethylthio-ester with Raney nickel gave 4-methylpent-2-enal; ethyl 4-methylpent-3-enoate when treated with sodium gave an impure acyloin which polymerised on distillation, and oxidation of the supposed acyloin or the corresponding glycol with periodate gave several products of which only acetone and 3-methylbut-2-enal could be characterised.

Ozonolysis of fumagillol in ethyl acetate followed by hydrogenation over palladium–calcium carbonate gave two crystalline products, an aldehyde, $C_{13}H_{20}O_5$,[6] and an acid, $C_{13}H_{20}O_6$, together with acetone [6] and much amorphous material probably arising from further degradation of the molecule. The ordinary conditions of ozonolysis involving prolonged treatment with ozonised oxygen may well lead to general oxidative degradation of an unstable molecule, but treatment of fumagillol with a solution of ozone (1 equivalent) in ethyl acetate did not give a better yield of aldehyde. The aldehydic ozonolysis product was shown to be αβ-unsaturated by its ultraviolet and infrared spectra, and like croton-aldehyde it liberated alkali slowly from sodium thiosulphate solution. It did not give a chlorohydrin with dilute hydrochloric acid. It was unstable to chromatography on alumina, and on oxidation with neutral or acid permanganate it consumed 4·5—5 atoms of oxygen. The C_{13} acid is presumably the one derived from the aldehyde by oxidation without other change of structure, but this has not been proved, and the infrared carbonyl frequency (1715 cm.$^{-1}$) is high for an αβ-unsaturated acid.

Ozonolysis of the C_{13} aldehyde under the conditions used for fumagillol gave a mixture which was partially separable by chromatography on silica, the main fraction being a weakly lævorotatory syrup. Methylglyoxal was also formed, and was identified by the melting point and ultraviolet absorption of its bis-2 : 4-dinitrophenylhydrazone; the infrared spectrum and *X*-ray powder photograph, however, showed small differences from those of authentic methylglyoxal bis-2 : 4-dinitrophenylhydrazone which could be attributed to the presence of a small amount of glyoxal bis-2 : 4-dinitrophenylhydrazone (Schenck *et al.*[6] obtained glyoxal on ozonolysis of the C_{13} aldehyde).

The splitting-off of a C_6 fragment by periodate oxidation after hydrolysis of the epoxide group shows that there are five carbon atoms attached to one side of the epoxide ring and not joined to the rest of the molecule in a cyclic structure (the formation of acetone in this experiment must be attributed to a side reaction). The formation of the αβ-unsaturated C_{13} aldehyde on ozonolysis involves the destruction of the epoxide group which must be so placed that it generates the new double bond. These results can be interpreted by the annexed scheme.

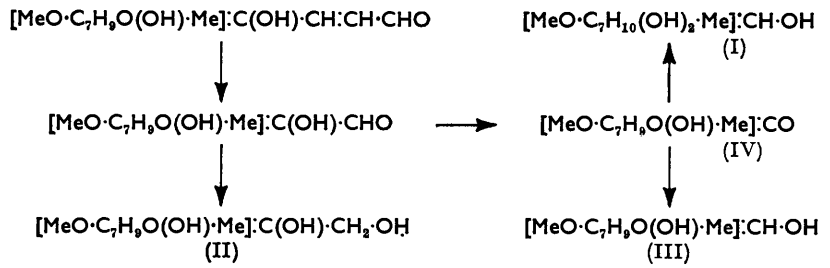


The formation of γ-methylvaleric acid in the oxidation of tetrahydrofumagillol [7] and the isolation of ethyl *iso*pentyl ketone on the dehydrogenation of hexahydrofumagillol [6] support this partial structure. The formation of methylglyoxal in the ozonolysis of the C_{13} aldehyde is an anomaly, but a C_{11} fragment can be isolated from this ozonolysis (see below) and on the present intepretation the methylglyoxal must arise from the disruption of some other part of the molecule. It may indicate that the methyl and the hydroxyl group are attached to the same carbon atom. The C_{13} aldehyde has a strong infrared band at approx. 980 cm.$^{-1}$, characteristic of a *trans*-disubstituted ethylene.

The part of the molecule not accounted for must be a saturated bicyclic structure, $C_9H_{16}O_3$, containing the hydroxyl and the methoxyl group, a *C*-methyl group, and the other oxygen atom, and unless the rings are small one of them must include a carbon atom of the epoxide group. Ozonolysis of the C_{13} aldehyde should give compounds with a carbonyl group directly attached to or incorporated in the fundamental ring system at the position of the original epoxide group.

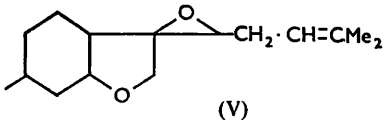
Reduction of the weakly lævorotatory ozonolysis product from the C_{13} aldehyde with

lithium aluminium hydride gave two crystalline alcohols, $C_{11}H_{20}O_5$ and $C_{10}H_{20}O_4$. These could not be obtained by similar reduction of the other fractions from the ozonolysis, of the permanganate oxidation product of the C_{13} aldehyde, of the periodate oxidation product of fumagillol, or of a substance obtained by oxidising the C_{13} acid with hydrogen peroxide–osmium tetroxide and then with periodate. A fraction from the ozonolysis of fumagillol which appeared (from infrared spectroscopy) to consist largely of a mixture of C_{13} aldehyde and C_{13} acid was ozonised and reduced with lithium aluminium hydride, giving another crystalline alcohol, $C_{10}H_{18}O_4$. The isolation of the C_{10} alcohols indicates that a formyl group has been lost during ozonolysis; similar reactions, for example, the formation of phenols during ozonolysis of some cinnamic esters,[10] have been reported. The simplest interpretation of these reactions is shown by the scheme below.

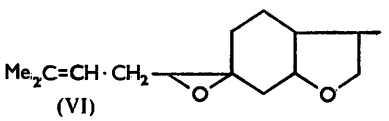


The formation of a compound $C_{10}H_{20}O_4$ must also involve the reductive fission of the remaining oxygen bridge; instances are known where lithium aluminium hydride splits a tetrahydrofuran ring, although more vigorous reaction conditions are usually needed. The *C*-methyl content of this alcohol indicates that no new methyl group has been generated unless it is in an *iso*propyl group. When the C_{10} and C_{11} alcohols were treated with periodic acid the compound $C_{10}H_{18}O_4$ consumed 0·15 mol. in three days and the others did not react. This unexpected result indicates that $C_{10}H_{20}O_4$ (I) cannot be a 1 : 2-glycol, which would be formed if the side chain is β to the oxygen bridge; and $C_{11}H_{20}O_5$ cannot be (II), but must be an isomer formed by migration of the nuclear hydroxyl group. The alcohol $C_{10}H_{18}O_4$ (III) was oxidised with chromic acid to a cyclic ketone, probably (IV). The crude product showed a single infrared carbonyl frequency at 1714 cm.$^{-1}$; this would have been displaced to *ca.* 1740 cm.$^{-1}$ if the carbonyl group was in a five-membered ring. Oxidation of $C_{11}H_{20}O_5$ with chromic acid might give a cyclic ketone through the complete removal of the side chain, but the observed carbonyl frequencies of the crude oxidation product (approx. 1706 and 1738 cm.$^{-1}$) are probably attributable to a ketone (not in a five-membered ring) and an aldehyde or ester. The oxidation product of $C_{10}H_{20}O_4$ showed strong absorption at the same frequencies, and the ozonolysis fraction from which the alcohols were obtained absorbed strongly at 1700 but not at 1740 cm.$^{-1}$.

From the selenium dehydrogenation of tetrahydrofumagillol Tarbell *et al.*[7] isolated a substance with chemical and physical properties resembling those of a benzofuran, and this suggests that the fundamental ring system is a furano*cyclo*hexane. In view of the formula of fumagillol, $C_{15}H_{23}O_3$·OMe, and the known structure of the side-chain, $Me_2C{:}CH{\cdot}CH_2$,



(V)



(VI)

it might well be related to the terpenes, and on this hypothesis partial formulæ such as (V) and (VI) deserve consideration. The data from the oxidation of the C_{10} and C_{11} alcohols cannot readily be explained unless the side chain is attached to a six-membered ring and is not α or β to the oxygen bridge. Structure (VI) is compatible with the

[10] Späth and Pailer, *Ber.*, 1940, **73**, 238; Späth, Pailer, and Gergely, *ibid.*, p. 795.

experimental results, but positive evidence for it is lacking. Attempts to obtain identifiable aromatic compounds, for example, by treating fumagillol or the C_{13} acid with concentrated hydrobromic or hydriodic acid, were inconclusive.

Experimental

Fumagillin was isolated from the metabolic products of *Aspergillus fumigatus* (strain A.61) as described by Goodall and Landquist,[11] and was purified by crystallisation from pentan-2-one.

Silica for chromatography was prepared as described by Martin and Synge.[12] Not all batches were suitable for separating the ozonolysis products of fumagillol, and a commercial brand of silica (Mallinckrodt) was also unsuitable for this purpose.

Hydrolysis of Fumagillin.—(*a*) Fumagillin (12·0 g.) was dissolved in 0·1N-sodium hydroxide (1·2 l.) under nitrogen, and after 4 hr. at room temperature the solution was filtered from a trace of flocculent material and extracted with ether (3 × 1 l.). Evaporation of the dried (Na_2SO_4) extract gave crude *fumagillol* (6·7 g.) which crystallised from light petroleum (b. p. 40—60°), giving 5·6 g., m. p. 54—56°, $[\alpha]_D^{24}$ −52° (1·27% in $CHCl_3$), b. p. 140°/0·02 mm. [Found: C, 68·0; H, 9·2; OMe, 10·9; OH (as active hydrogen), 6·7; *C*-Me, 7·1. $C_{16}H_{26}O_4$ requires C, 68·0; H, 9·2; OMe, 11·0; OH, 6·0; *C*-Me, 5·3% per Me group]. Evaporation of the petroleum mother-liquor gave syrupy fumagillol (0·8 g.), $[\alpha]_D$ *ca.* −40°. Re-extraction of the hydrolysis solution with ether after 20 hr. gave a trace more fumagillol (*ca.* 0·2 g.), and acidification precipitated octatetraene-1 : 8-dicarboxylic acid (4·6 g.).

(*b*) Fumagillin (10 g.), methanol (1 l.), and 40% aqueous sodium hydroxide (100 c.c.) were boiled under reflux for 1·5 hr., then cooled, and sodium octatetraene-1 : 8-dicarboxylate was filtered off. The filtrate was evaporated under reduced pressure at 30° and the residue was dissolved in water and extracted with ether (3 × 350 c.c.). Evaporation of the dried (Na_2SO_4) extract gave a syrupy *product*, $[\alpha]_D$ −33°, which was distilled at 0·005 mm. (bath-temp. 160—180°) (Found: C, 65·7; H, 10·3; OMe, 18·35, 18·5. $C_{17}H_{30}O_5$ requires C, 65·0; H, 9·55; 2OMe, 19·8%).

(*c*) Fumagillin (6·0 g.) in methanol (500 c.c.) was treated at 0° with 40% aqueous sodium hydroxide (5·0 c.c.). After 16 hr. at room temperature, the sodium salt was collected and dissolved in water, and the solution acidified to precipitate methyl hydrogen octatetraene-1 : 8-dicarboxylate (1·05 g.), m. p. 216—218°. Evaporation of the methanolic solution and extraction of the residue as in the foregoing experiment gave a syrup (3·1 g.), $[\alpha]_D$ −47° (Found: C, 65·6; H, 9·2; OMe, 18·3%).

Hydrogenated Derivatives.—(*a*) Fumagillol (0·75 g.) in water (100 c.c.) was hydrogenated over Adams platinum oxide catalyst, absorbing *ca.* 62 c.c. of hydrogen at N.T.P. in 30—35 min. (required for 1 mol. on M = 282, 59 c.c.). The filtered solution was extracted with ether, and the dried extract evaporated, giving *dihydrofumagillol* (0·57 g.) as a viscous syrup (Found: C, 69·5; H, 9·2; OMe, 10·3. $C_{16}H_{28}O_4$ requires C, 67·5; H, 9·85; OMe, 10·9%).

(*b*) Fumagillin (1·5 g.) in methanol (150 c.c.) was hydrogenated over platinum oxide, taking up 360 c.c. at N.T.P. in 45 min. (required for 5 mols., 375 c.c.). The filtered solution was evaporated under reduced pressure, giving a colourless syrup (1·4 g.; $[\alpha]_D$ −24° in MeOH) which slowly became jelly-like and showed signs of crystallising at the surface. Decahydrofumagillin (2·0 g.) was dissolved in 0·1N-sodium hydroxide (200 c.c.), and after 18 hr. at room temperature the solution was filtered and extracted with ether (4 × 200 c.c.). Acidification of the aqueous layer precipitated sebacic acid (0·76 g.), m. p. 133°. Evaporation of the dried (Na_2SO_4) extract gave dihydrofumagillol as a syrup (Found: C, 65·6; H, 9·7; OMe, 10·4%).

Functional Derivatives of Fumagillol.—Attempts to characterise fumagillol by treating it with 3 : 5-dinitrobenzoyl chloride, α-naphthyl *iso*cyanate, or fluoro-2 : 4-dinitrobenzene by standard procedures were unsuccessful. Keten did not acetylate fumagillol, and treatment with acetyl chloride and triethylamine in toluene gave a syrup, b. p. 165—170°/0·02 mm., which was not the required acetate (Found: C, 68·3; H, 8·7; OMe, 11·7; OAc, 16·7. Calc. for $C_{18}H_{28}O_5$: C, 66·6; H, 8·6; OMe, 9·55; OAc, 13·3%).

Oppenauer Oxidation.—Fumagillol (2·0 g.), aluminium *tert.*-butoxide (4·0 g.), freshly distilled *cyclo*hexanone (100 c.c.), and dry toluene (100 c.c.) were boiled under reflux for 3 hr., cooled, washed with ice-cold dilute hydrochloric acid and with water, dried (Na_2SO_4), and evaporated

[11] Goodall and Landquist, *Analyst*, 1955, **80**, 499.
[12] Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.

under reduced pressure. Distillation at 0·02 mm. afforded a viscous yellow oil (1·1 g.) with a strong floral odour, b. p. 145—150° (Found: C, 67·6; H, 9·1; OMe, 9·2%). This product had only a weak absorption band at 1705 cm.$^{-1}$.

Reactions Characteristic of the Epoxide Group.—(*a*) Fumagillol (0·25 g.), dissolved in water (22·5 c.c.), was treated with N-hydrochloric acid (2·5 c.c.). The solution rapidly became turbid, and after 24 hr. it was extracted with ether (3 × 25 c.c.) and the extract was dried (Na_2SO_4) and evaporated. The syrupy chlorohydrin (0·15 g.) had $[\alpha]_D^{24}$ +29·4° (1·5% in $CHCl_3$) (Found: C, 56·0; H, 7·9; OMe, 9·3, 9·5; Cl, 7·7; active H, 0·52. Calc. for $C_{16}H_{27}O_4Cl$: C, 60·2; H, 8·46; OMe, 9·7; Cl, 11·15; active H, 0·314% per OH group). A sample obtained after a more prolonged reaction was distilled, b. p. 170°/0·02 mm. (Found: C, 58·8; H, 8·4; Cl, 11·3%).

(*b*) Aqueous solutions of fumagillol and sodium thiosulphate mixed at room temperature rapidly became alkaline to phenolphthalein. Fumagillol (1·5 g.), sodium thiosulphate (4·0 g.), and water (100 c.c.) were heated at 40—50° and kept just acid to phenolphthalein by titration with 0·212N-acetic acid. The end-point (23·1 ml.) was reached in about 40 min. (calc. for 1 epoxide group 25·0 ml.). The mixture was made strongly alkaline with sodium hydroxide, heated at 40° for 15 min., cooled, and extracted with ether (8 × 120 c.c.). The dried (Na_2SO_4) extract was evaporated under reduced pressure, giving the disulphide as a glass (Found: C, 60·1; H, 8·7; S, 8·8; OMe, 9·0. $C_{32}H_{54}O_8S_2$ requires C, 60·8; H, 8·55; S, 10·15; OMe, 9·8%). In a subsequent experiment, the alkaline aqueous reaction mixture deposited a trace of crystalline material on storage. This formed crystals, m. p. 152—153°, from aqueous ethanol (Found: C, 60·6; H, 7·9; S, 9·9%). The infrared spectrum did not show thiol absorption.

(*c*) Fumagillol (0·89 g.), magnesium bromide–diether complex (1·05 g.), and dry ether were boiled under reflux overnight. There was no loss of the epoxide reaction with thiosulphate. The ether was replaced by dibutyl ether and refluxing was continued for 6 hr., whereafter epoxide could no longer be detected. Water and ether were added to dissolve the magnesium bromide and the products of reaction, and the ether layer was separated, dried (Na_2SO_4), and evaporated under reduced pressure, leaving a light brown syrup, $[\alpha]_D$ −29° (in $CHCl_3$), which showed infrared carbonyl absorption.

(*d*) Lithium aluminium hydride (1·0 g.) and dry ether (50 c.c.) were boiled under reflux for 2 hr., cooled, and treated gradually with fumagillol (1·0 g.) in dry ether (25 c.c.). The mixture was boiled for 3 hr., left overnight, and decomposed cautiously with water. The aqueous layer was separated and extracted with ether, and the combined ether solutions were dried (Na_2SO_4) and evaporated, giving a syrup which did not show an epoxide reaction with thiosulphate (Found: C, 66·1; H, 10·0; OMe, 10·8. Calc. for $C_{16}H_{28}O_4$: C, 67·5; H, 9·85; OMe, 10·9. Calc. for $C_{16}H_{30}O_4$: C, 67·1; H, 10·5; OMe, 10·8%). A similar product was obtained by desulphurising the disulphide (0·9 g.) with Raney nickel (5 g.) in boiling ethanol (30 c.c.) (Found: C, 65·3; H, 9·9; OMe, 11·0%).

(*e*) Fumagillol (0·5 g.) and N-sodium hydroxide (10 c.c.) were heated on the steam-bath until a neutralised sample no longer gave a reaction with sodium thiosulphate (48—64 hr.). The syrupy product (0·41 g.) was recovered by extraction with ether and dried at 90—100°/10 mm. (Found: C, 65·8, 65·6; H, 9·3, 8·9; OMe, 10·4. Calc. for $C_{16}H_{28}O_5$: C, 64·0; H, 9·33; OMe, 10·33%).

(*f*) Fumagillol (0·5 g.) and N/50-sulphuric acid (50 c.c.) were heated on the steam-bath until the epoxide reaction had disappeared (½—2 hr.), and the product (0·3 g.) was extracted with ether (Found: C, 63·5; H, 8·9; OMe, 11·1%). This material distilled mainly at 180°/0·01 mm. A similar hydrolysis carried out at room temperature took *ca.* 24 hr. for completion, and gave a product with a less complex infrared spectrum (Found: C, 60·2; H, 8·9; OMe, 11·3%).

Periodate Oxidation of Glycol from Treatment of Fumagillol with Cold 0·02N-Sulphuric Acid.—The glycol (0·8 g.), potassium metaperiodate (1·3 g.), and water (50 c.c.) were acidified with sulphuric acid and heated at 90—100°. Air was aspirated through the mixture and then through a saturated solution of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid. After 3—4 hr. the precipitated 2 : 4-*dinitrophenylhydrazone* was collected, washed with dilute hydrochloric acid and water, dried, and crystallised from *cyclo*hexane. The crystals (40 mg.), m. p. 116—122°, were purified by chromatography on silica, with elution by *cyclo*hexane–benzene, and the main band was crystallised three times from *cyclo*hexane, giving 4 mg., m. p. 156—157° (Found: C, 51·4; H, 5·3. $C_{12}H_{14}O_4N_4$ requires C, 51·8; H, 5·0%). In a later experiment the crude 2 : 4-dinitrophenylhydrazone (48 mg.) was separated by chromatography on bentonite–kieselguhr [13] into acetone 2 : 4-dinitrophenylhydrazone (5 mg.), m. p. and mixed m. p. 123—125°, and the C_6 2 : 4-dinitrophenylhydrazone (11 mg.), m. p. 159—160° (Found: C, 52·3; H,

[13] Elvidge and Whalley, *Chem. and Ind.*, 1955, 589.

5·1%), ultraviolet absorption max. at 363 mμ (ε 21,900 in EtOH). When fumagillol was treated with periodic acid under the same conditions, only acetone 2 : 4-dinitrophenylhydrazone was isolated.

Ozonolysis of Fumagillol.—Fumagillol (5 g.), dissolved in dry ethyl acetate (100 c.c.), was cooled to −70° and treated with ozonised oxygen (*ca.* 7·5 l./hr.) until the solution became faintly blue and ozone issued freely from the reaction vessel. Excess of ozone was removed under reduced pressure and the solution was hydrogenated over pre-reduced palladium–calcium carbonate. The reaction was slow and the hydrogen uptake variable. The filtered solution was evaporated and acetone was isolated from the volatile portion as its 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 126—127°. The residual gum was dissolved in ethyl acetate–benzene and chromatographed on 20 parts of silica. Elution with ethyl acetate–benzene gave two fractions, $[\alpha]_D$ +10—50° (2—3 g.) and $[\alpha]_D$ −20° to −30° (0·2—0·3 g.). A third fraction, $[\alpha]_D$ *ca.* −40° (0·2—0·9 g.) was eluted with ethyl acetate, and a fourth (0·3—1·4 g.) with ethanol. Crystallisation of the first fraction from ethyl acetate–light petroleum (b. p. 40—60°) gave the C_{13} aldehyde (0·8—1·8 g.), m. p. 153—155°, $[\alpha]_D$ +63° (1% in ethyl acetate), ultraviolet absorption max. at 220 mμ, infra-red absorption at 1685 cm.$^{-1}$ (very strong) (Found : C, 60·7; H, 7·5; OMe, 11·9. Calc. for $C_{13}H_{20}O_5$: C, 60·9; H, 7·8; OMe, 12·1%). Fractions 2 and 3 were mixtures of aldehyde and acid (infrared absorption at 1685 and 1715 cm.$^{-1}$), and fraction 3 slowly deposited crystals of the C_{13} *acid* which, crystallised from ethyl acetate–light petroleum, had m. p. 143—144°, $[\alpha]_D$ −65° (1·7% in ethyl acetate) (Found : C, 57·2; H, 7·2; OMe, 11·6%; equiv., 259, 266. $C_{13}H_{20}O_6$ requires C, 57·35; H, 7·35; OMe, 11·4%; equiv., 272). Fraction 4 was a brown gum, $[\alpha]_D$ *ca.* −35°. An inefficient separation of aldehyde was achieved by extracting the ethyl acetate solution of the ozonolysis products with aqueous sodium hydrogen carbonate. Crystalline aldehyde was recovered by evaporation of the ethyl acetate, but a considerable proportion was extracted into the aqueous layer with the acid and other by-products.

Ozonolysis of the C_{13} Aldehyde.—The aldehyde (2·0 g.) in dry ethyl acetate (100 c.c.) was ozonised at −70° until a faint blue colour indicated an excess of ozone. This was removed by aspirating air through the solution, and the ozonide was hydrogenated over palladium–calcium carbonate (very slow absorption of *ca.* 70 c.c. of hydrogen). The filtered solution was evaporated and the residue was heated at 60—65°/10 mm., the vapour passing through 2 : 4-dinitrophenylhydrazine hydrochloride solution. The latter gave a red-orange precipitate, m. p. 245—268°, which was crystallised twice from pyridine to give methylglyoxal bis-2 : 4-dinitrophenylhydrazone,[14] m. p. and mixed m. p. 294—296°. The non-volatile ozonolysis product (1·91 g.) was chromatographed in ethyl acetate–benzene on silica (45 g.), giving as successive fractions 0·13 g. of $[\alpha]_D$ −12°, 0·10 g. of $[\alpha]_D$ −10°, 0·09 g. of $[\alpha]_D$ −3°, 0·13 g. of $[\alpha]_D$ −19°, and, on change of solvent to pure ethyl acetate, 0·2 g. of $[\alpha]_D$ −9·5° and 0·73 g. of $[\alpha]_D$ −15° (all syrups or gums). This result was fairly reproducible, a better yield of the first fractions sometimes being obtained. The tail fraction resembled that from the ozonolysis of fumagillol in showing marked infrared absorption at *ca.* 1600 cm.$^{-1}$, and a broad carbonyl band at *ca.* 1720 cm.$^{-1}$. The first lævorotatory fractions, $[\alpha]_D$ *ca.* −10° (Found : C, 58·5, 58·2; H, 7·7, 7·9%), showed a narrow and intense infrared band at about 1700 cm.$^{-1}$ and appeared to be the most homogeneous of the ozonolysis products.

Reduction of the Ozonolysis Product.—The material (0·69 g.), $[\alpha]_D$ *ca.* −10°, from the ozonolysis of C_{13} aldehyde, lithium aluminium hydride (2·0 g.), and dry ether (150 c.c.) were boiled under reflux for 2 hr., left at room temperature overnight, treated cautiously with water, and just acidified with dilute sulphuric acid. The mixture was extracted with ether (3 × 150 c.c.), and the extract was dried (Na_2SO_4) and evaporated. The residue gradually afforded crystals (0·19 g.; m. p. 130—133°) which were collected, washed with ether, and crystallised twice from ethyl acetate to give an *alcohol* (0·04 g.), m. p. 162—164°, $[\alpha]_D$ −60° (0·8% in ethyl acetate) (Found : C, 58·5; H, 9·9; *C*-Me, 6·3. $C_{10}H_{20}O_4$ requires C, 58·8; H, 9·8; *C*-Me, 7·35%). The material in the mother-liquor and in the ethereal washings was partly separated by chromatography on silica or alumina (with ethyl acetate–benzene) into the compound of m. p. 162—164° and an *alcohol*, m. p. 121—122°, $[\alpha]_D$ −71° (in ethyl acetate) (Found : C, 57·4; H, 9·4. $C_{11}H_{20}O_5$ requires C, 56·8; H, 8·6%). Because of the inefficiency of the separation, the relative proportions of the two compounds could not be ascertained.

A similar reduction carried out on the corresponding first fractions (1·18 g.) from the ozonolysis of a mixture of C_{13} aldehyde and C_{13} acid (fraction 2 of the ozonolysis products of fumagillol) gave an uncrystallisable syrup (0·67 g.), $[\alpha]_D$ −18°, which was dissolved in ethyl

[14] Bülow and Seidel, *Annalen*, 1924, **439**, 55.

acetate–benzene, adsorbed on silica (12 g.), and eluted with ethanol. The fractions which crystallised on evaporation (0·06 g.) were recrystallised from ethyl acetate–light petroleum (b. p. 40—60°), forming long prisms (0·027 g.) of a *product*, m. p. 124—126°, $[\alpha]_D$ −42° (in ethyl acetate) (Found: C, 59·5; H, 8·9. $C_{10}H_{18}O_4$ requires C, 59·4; H, 8·9%).

Oxidation of the C_{10} *and the* C_{11} *Alcohol.*—(*a*) The alcohol, $C_{10}H_{20}O_4$, m. p. 162—164° (5 mg.), in water (0·5 c.c.) was treated with 0·35 c.c. of a solution of chromium trioxide (1 g.) and sulphuric acid (1·5 g.) in water (50 c.c.). The mixture was shaken overnight and then extracted continuously with ether for 24 hr. Evaporation of the dried (Na_2SO_4) extract gave an oil (3·4 mg.). The other alcohols, $C_{11}H_{20}O_5$ (m. p. 121—122°) and $C_{10}H_{18}O_4$ (m. p. 124—126°), were oxidised similarly. The crude products were used for infrared spectroscopy.

(*b*) Periodate oxidations were studied with 2 mg. samples of the three alcohols, the periodate concentration being determined at intervals by ultraviolet spectroscopy.[15] In neutral solution no periodate was taken up in 3 hr.; in acid solution the alcohol $C_{10}H_{18}O_4$ consumed 0·15 mol. in 3 days and the others did not consume any.

N-*Methyl*-4-*methylpent*-3-*enanilide*. Methylaniline (15 g.) in dry ether (25 c.c.) was added to an ice-cold solution of methylmagnesium iodide (from 19·9 g. of methyl iodide and 3·4 g. of magnesium) in ether (100 c.c.). The mixture was stirred for 15 min. and then treated with ethyl 4-methylpent-3-enoate (9·8 g.) in ether (20 c.c.), stirred for 2·5 hr. at room temperature, and acidified with 2N-hydrochloric acid. The ether layer was separated, washed with water (2 × 25 c.c.), dried (Na_2SO_4), and distilled, giving the *product* (6·4 g.) as a yellow oil, b. p. 95—99°/0·25 mm. (Found: C, 76·6; H, 8·2; N, 7·4, 6·5. $C_{13}H_{17}ON$ requires C, 76·85; H, 8·35; N, 6·9%). The methylanilide (3 g.) in ether (30 c.c.) was stirred with lithium aluminium hydride (0·14 g.) at 0° for 21 hr., and the carbonyl compounds produced were isolated as 2 : 4-dinitrophenylhydrazones. Chromatography of these on bentonite–kieselguhr showed the presence of at least four compounds in amounts insufficient for characterisation.

Acyloin from Ethyl 4-*Methylpent*-3-*enoate*.—Ethyl 4-methylpent-3-enoate (22·3 g.) was added during 1 hr. to powdered sodium (7·23 g.) in ether (120 c.c.), and the mixture was stirred and boiled under reflux until almost all the sodium had dissolved (24 hr.). The mixture was then cooled in ice and made faintly acid by the careful addition of 50% sulphuric acid. The ether layer was separated, washed with aqueous sodium carbonate, dried (Na_2SO_4), and evaporated. Distillation of the residue gave 0·8 g. of b. p. 60—100°/12 mm., 1·8 g. of b. p. 120—150°/12 mm., 1·4 g. of b. p. 160—200°/12 mm., and a considerable residue. Re-distillation of the second fraction gave 0·6 g., b. p. 120—130°/12 mm. (Found: C, 72·6; H, 10·0. Calc. for $C_{12}H_{20}O_2$: C, 73·47; H, 10·2%), and 0·1 g., b. p. 130—140°/12 mm. The use of xylene [16] instead of ether was not advantageous. The acyloin had infrared absorption bands at 3450 and 1710 $cm.^{-1}$.

Periodate Oxidations.—The foregoing acyloin fraction of b. p. 120—130°/12 mm. (0·4 g.) was reduced with lithium aluminium hydride (0·25 g.) in ether, and the glycol (0·4 g.) recovered from the ether after acidification was treated with periodic acid (2 g.) in water (100 c.c.) at 90—100°. Air was aspirated through the hot solution and then through a solution of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid. The dinitrophenylhydrazones were collected after several hours and separated by chromatography on bentonite–kieselguhr. The main fractions were acetone 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 126—127°, and 3-methylbut-2-enal 2 : 4-dinitrophenylhydrazone, m. p. 176—178°, ultraviolet absorption max. at 380 mμ (ε 27,400 in EtOH) (Found: C, 49·5; H, 4·7; N, 20·6. Calc. for $C_{11}H_{12}O_4N_4$: C, 49·8; H, 4·8; N, 21·2%). Braude and Jones [17] found the m. p. to be 179°, but higher values have been recorded. The same dinitrophenylhydrazones were isolated from periodate oxidation of other fractions from the acyloin condensation (without previous reduction with lithium aluminium hydride).

Ethyl 4-*Methylpent*-3-*enthiolate*.—Ethanethiol (3·6 g.), pyridine (5·0 g.), and benzene (15 c.c.) were stirred and treated with 4-methylpent-3-enoyl chloride (7·7 g.) in benzene (15 c.c.) at <30°. After 1 hr. pyridine hydrochloride was filtered off and the filtrate was washed with water, dried (Na_2SO_4), and distilled. The *thiol ester* (2·5 g.) boiled at 85—90°/16 mm. (Found: C, 60·3; H, 8·6. $C_8H_{14}OS$ requires C, 60·76; H, 8·85%).

Desulphurisation. Raney nickel (10 g.) was stirred under boiling acetone (50 c.c.) for 2 hr. Ethyl 4-methylpent-3-enthiolate (1·0 g.) was added and the mixture was stirred under reflux for 1 hr., filtered from nickel, and evaporated. The residual oil was suspended in warm water

[15] Dixon and Lipkin, *Analyt. Chem.*, 1954, **26**, 1092; Marinetti and Rouser, *J. Amer. Chem. Soc.*, 1955, **77**, 5345.
[16] Hansley, *J. Amer. Chem. Soc.*, 1935, **57**, 2303.
[17] Braude and Jones, *J.*, 1945, 498.

(50 c.c.), and air was drawn through the mixture to entrain first acetone and then 4-methylpent-2-enal, which was isolated as its 2 : 4-*dinitrophenylhydrazone*, scarlet needles, m. p. 203—204°, from ethyl acetate (Found : C, 51·3; H, 5·0; N, 19·7. $C_{12}H_{14}O_4N_4$ requires C, 51·8; H, 5·0; N, 20·1%), ultraviolet absorption max. at 381 mμ (ε 23,000 in EtOH).

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