was heated at 100° (0.1 mm.) whereupon 0.336 g. (67.2%) of XVIII was collected as sublimate. Evaporation at room temperature and atmospheric pressure of the 300 ml. of distillate from the steam distillation left no trace of XVIII. A non-distillable residue of 0.080 g. remained in the sublimation tube.

α,α,α-Tri-(γ-bromopropyl)-acetic Acid (XX).—To a solution of 0.500 g. of XVIII in 30 ml. of anhydrous ether was added 70 ml. of a saturated solution of hydrogen bromide in anhydrous ether. After standing overnight at room temperature, the ether and hydrobromic acid were evaporated in a stream of air to approximately 30 ml., which was shaken with water until the washings were no longer acidic. After removal of the ether at reduced pressure, the remaining oil was dissolved in hot petroleum ether (b.p. 60–68°). Upon cooling, 0.561 g. (52.5%) of colorless crystals, m.p. 90.5–92°, precipitated; after two recrystallizations the m.p. was 93–94°.

Anal. Calcd. for $C_{11}H_{19}O_2Br_3$: C, 31.23; H, 4.53. Found: C, 31.73; H, 4.44.

MADISON, WISCONSIN

[CONTRIBUTION FROM ABBOTT LABORATORIES]

Chemistry of Fumagillin. II[1]

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The alcohol portion ($C_{16}H_{26}O_4$) of fumagillin ($C_{26}H_{34}O_7$) can be ozonized to yield a crystalline α,β-unsaturated aldehyde ($C_{13}H_{20}O_5$) and acetone. The dihydroalcohol reacts with dinitrobenzoyl chloride to yield a crystalline chlorine-containing dinitrobenzoate ($C_{23}H_{31}N_2O_9Cl$). The hexahydroalcohol reacts with dinitrobenzoyl chloride to form a bis-dinitrobenzoate ($C_{30}H_{36}N_4O_{14}$). Dehydrogenation of the hexahydroalcohol yields ethyl isoamyl ketone and aromatic products. Oxidation of the dihydroalcohol yields succinic acid.

Fumagillin[1-3] has the empirical formula $C_{26}H_{34}O_7$ and is a mono-ester of decatetraenedioic acid and a neutral substance $C_{16}H_{26}O_4$, which we have called alcohol I. Analyses of fumagillin itself as well as alcohol I indicate that the alcohol must contain one methoxyl, one hydroxyl, two C-methyl groups and two non-carbonyl oxygen atoms.

Ozonolysis.—When alcohol I[4] is ozonized, it is split into acetone and a crystalline α,β-unsaturated aldehyde $C_{13}H_{20}O_5$. The reaction of this ozonolysis product with Tollens reagent, its ultraviolet spectrum and the spectrum of its thiosemicarbazone are all in agreement with its formulation as an α,β-unsaturated aldehyde. Since alcohol I shows no evidence of a conjugated system, the carbon–carbon double bond must have arisen by opening of an oxygen bridge or by migration of another double bond. The first hypothesis explains the appearance of both the double bond and an extra hydroxyl group. Further ozonolysis yields glyoxal.

CHART I

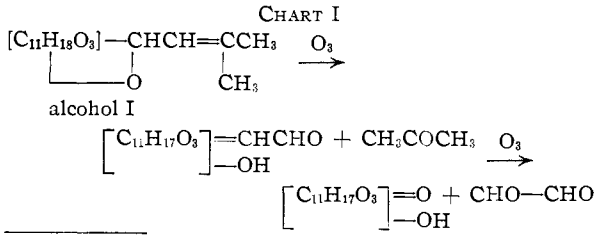


Chart I indicates one possible course of this reaction.

Hydrogenation.—The alcohol portion of fumagillin forms a series of hydrogenation products in which one, two and three moles of hydrogen are added. It is believed that only one double bond is involved and that the other two moles of hydrogen enter by hydrogenolysis of oxygen bridges. The elementary composition of alcohol I is in agreement with the presence of three rings and one double bond. A dihydroalcohol may be prepared by hydrogenation of fumagillin, followed by saponification. A crystalline tetrahydroalcohol[5] has been obtained by hydrogenation of alcohol I. A hexahydroalcohol has been prepared by hydrogenation of the tetrahydroalcohol in glacial acetic acid or by hydrogenation of fumagillin in glacial acetic acid, followed by saponification.

The preparations of the dihydroalcohol made either by hydrogenation of fumagillin followed by saponification or by saponification of fumagillin followed by hydrogenation usually contained a considerable amount of hydrated material as shown by low carbon and high oxygen analyses. A crystalline derivative was obtained by reaction with dinitrobenzoyl chloride. Analyses disclosed that this compound was not a normal dinitrobenzoate but contained chlorine and had the composition ($C_{23}H_{31}N_2O_9Cl$) corresponding to addition of one mole of dinitrobenzoyl chloride to one mole of the dihydroalcohol. Active hydrogen determinations indicated that two hydroxyl groups remained. The presence of chlorine indicates that the reaction involves addition, probably by cleavage of an oxygen bridge. The chlorine atom is inert; it does not react with silver nitrate.

The hexahydroalcohol reacts with dinitrobenzoyl chloride forming a crystalline bis-dinitrobenzoate ($C_{30}H_{36}N_4O_{14}$) which contains no chlorine, and the infrared spectrum indicates absence of hydroxyl groups.

The three hydrogenation products, according to our hypothesis of hydrogenation of one double

(1) Previous publication, J. R. Schenck, M. P. Hargie, D. S. Tarbell and P. Hoffman, THIS JOURNAL, **75**, 2274 (1953). Part of this material was presented before the Division of Medicinal Chemistry at the American Chemical Society Meeting in New York, September, 1954; D. S. Tarbell, H. R. Al-Kazimi, P. Hoffman, G. A. Page, H. R. Vogt, J. R. Schenck, M. P. Hargie and A. Isarasena, Abstracts of New York Meeting, 1954, p. 17 N.

(2) T. E. Eble and F. R. Hanson, *Antibiotics and Chemotherapy*, **1**, 54 (1951).

(3) C. J. Brown and J. K. Landquist, *Chemistry & Industry*, 973 (1953).

(4) Our preparations of alcohol I were probably not as pure as those of Tarbell,[5] *et al.*, and we have generally used the crude neutral fraction from saponification of fumagillin for ozonolysis.

(5) Paper III of this series: D. S. Tarbell, P. Hoffman, H. R. Al-Kazimi, G. A. Page, J. M. Ross, H. R. Vogt and B. Wargotz, THIS JOURNAL, **77**, 5610 (1955).

bond, followed by hydrogenolysis of two cyclic ether linkages, should show one, two and three hydroxyl groups, respectively. The alcohols themselves do have roughly the correct active hydrogen for this interpretation. The dinitrobenzoates obtained from the dihydro- and hexahydroalcohols, however, do not have the expected active hydrogen. The chlorodinitrobenzoate of the dihydroalcohol has two active hydrogens instead of the expected one. The bis-dinitrobenzoate of the hexahydroalcohol shows no free hydroxyl, according to the infrared spectrum, whereas one would be expected. No explanation of these unexpected results is offered although it must be admitted that neither of the alcohols could be considered pure.

Periodate treatment of the chlorodinitrobenzoate of the dihydroalcohol did not cause oxidation, so it may be concluded that the two hydroxyl groups are not adjacent. Hydrogen peroxide oxidation forms a product whose analyses correspond to a compound with two less carbon atoms.

Dehydrogenation.—The hexahydroalcohol has been dehydrogenated with selenium and with palladium-on-charcoal. A volatile product was collected and from it the dinitrophenylhydrazone of ethyl isoamyl ketone was isolated. The isolation of this compound confirms the presence of a branched side chain which the isolation of acetone and isocaproic acid[5] already have indicated. From the non-volatile products of dehydrogenation, two compounds have been isolated, each of which has lost the methoxyl group. One has peaks at 255 and 285 mμ in the ultraviolet and 6.13, 6.21, 6.30 and 6.73 μ in the infrared double bond region. Its analyses correspond to $C_{15}H_{18-20}O$. It is thought that this might be a benzofuran although two model benzofurans show no peak at 6.13 μ.

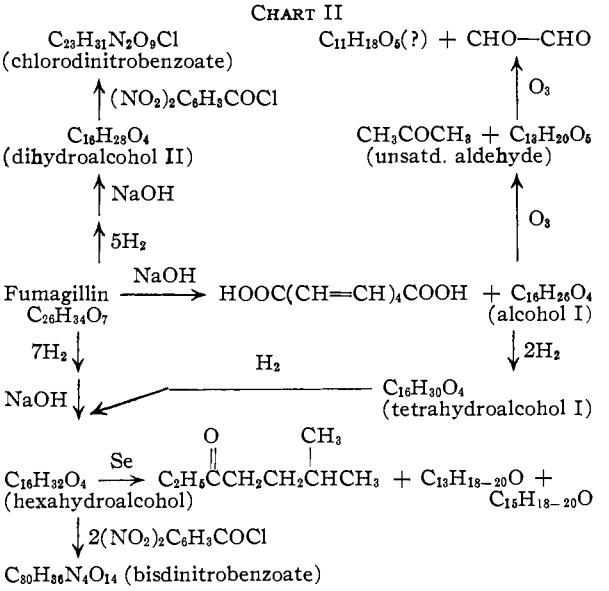
The second compound showed a peak in the ultraviolet at about 218 mμ and a carbonyl absorption band in the infrared at 5.84 mμ and double bond absorption at 6.24 and 6.31 μ. These infrared bands are the same for benzaldehyde, but attempts to isolate an acidic oxidation product were unsuccessful. Analyses for this compound corresponded to $C_{13}H_{18-20}O$.

Both of these compounds are aromatic and both have lost the methoxyl group as well as the hydroxyl groups which were present in the hexahydroalcohol. It appears likely that the hexahydroalcohol contained a six-membered ring which was aromatized by selenium.

Oxidation Products.—When the dihydroalcohol was oxidized with permanganate, a mixture of acids was obtained. Intensive efforts to separate and purify these led to the positive identification of only one minor component, which was succinic acid. Nitric acid oxidation led to a mixture of acids and oxalic was the only one positively identified. The fact that the nitric acid oxidation product still contains methoxyl is good evidence that the methoxyl could not be present as an enol-ether, ester or other acid labile group.

Summary.—Although the experiments described do not permit assignment of even a tentative formula for the alcohol portion of fumagillin, it may be concluded that a branched unsaturated side chain, as shown in Chart I, a six-membered carbocyclic ring, and perhaps two oxygen containing rings are present. Chart II summarizes the principal reactions described in this paper.

CHART II



Experimental

Fumagillin.—Crude commercial fumagillin of 75–90% purity was generally purified by dissolving 100 g. in 1 l. of chloroform and passing over a column of 50 g. of silica gel.[6] The first 2–3 l. of filtrate contained the fumagillin and much tarry material was removed by the silica gel. The chloroform was evaporated and the oil dissolved in warm amyl acetate. The fumagillin crystallized in 75–80% yield on cooling, with a purity of 90–95%. This was recrystallized by dissolving about 35 g. per l. in boiling methanol with addition of about 0.5 as much Darco G-60 as fumagillin. The hot solution was filtered and on cooling about 42% of the original crude fumagillin was recovered as white crystals of 95–98% purity. Over-all recovery of 73% could be obtained by adding water to the methanol mother liquors and by reworking the amyl acetate mother liquors.

Anal. Typical preparations. Found: C, 68.1 ± 0.3; H, 7.4 ± 0.2; O, 24.5 ± 0.3; OCH_3, 7.0 ± 0.3; C-methyl, 6.0 ± 0.5. Calcd. for $C_{26}H_{34}O_7$: C, 68.10; H, 7.48; O, 24.43; OCH_3, 6.77; C-methyl (2), 6.56.

The infrared spectrum shows no alcoholic OH absorption. Pure fumagillin has absorptivity, a = 156.0 at 335 mμ and 146.5 at 351 mμ[7] in solution made by dissolving 100 mg. in 10 ml. of chloroform and diluting with alcohol to a final concentration of 0.0004% fumagillin and 0.04% chloroform. Care should be taken that the alcohol (3A or 95%) contains no acid. Fumagillin is best stored in dark bottles in the absence of oxygen at low temperatures. It loses its activity against *E. histolytica*[8] and *Staph. phage*[9] as well as its ultraviolet absorption over a period of months when precautions are not taken. An excellent study of degradation of crystalline fumagillin was made by Garrett and Eble.[10]

Saponification of Fumagillin.—Fumagillin (18 g.) was dissolved in 800 ml. of 0.1 *N* NaOH and heated to 90°. On standing, it gradually became turbid; in 45 minutes, it was 70°; cooled and extracted continuously with ether

(6) G. F. Smith Chemical Co., Columbus, Ohio. Mr. J. E. Philip first developed this procedure.

(7) Spectrophotometric terms conform to "Suggested Nomenclature in Applied Spectroscopy," H. K. Hughes, *Anal. Chem.*, **24**, 1349 (1952).

(8) M. C. McCowen, M. E. Callender and J. F. Lawlis, Jr., *Science*, **113**, 202 (1951).

(9) F. R. Hansen and T. E. Eble, *J. Bact.*, **58**, 527 (1949).

(10) T. E. Eble and E. R. Garrett, *J. Am. Pharm. Assoc.*, **43**, 536 (1954); E. R. Garrett, *ibid.*, **43**, 539 (1954).

for 20 hours. (Several simple extractions were later found to be sufficient.) A pale yellow oil (10.1 g.) was obtained by evaporation. Similar preparations in this paper will be called alcohol I. Purified alcohol I is described by Tarbell[5] in paper III of this series. Decatetraenedioic acid could be recovered from the aqueous raffinate by acidification.

Ozonolysis of Alcohol I.—Alcohol I (10.1 g.) was dissolved in 100 ml. of chloroform. Ozonized oxygen was passed through the solution at 1 p.s.i. for 2.5 hr. Effluent gases were trapped in a solid carbon dioxide bath. The chloroform solution was warmed with portions of Raney nickel (total 15 g.) until a test with starch–KI paper was faint. The green solution was distilled and the chloroform trapped in an ice-bath. The oily residue was taken up in 25 ml. of ethyl acetate. Crystals formed immediately. These were centrifuged and then filtered to give 4.6 g. of a light green solid. This was dissolved in 50 ml. of acetone to separate an insoluble green residue. The acetone was evaporated and the residue (3.5 g.) dissolved in 60 ml. of hot ethyl acetate, the solution filtered and cooled. Crystals (1.96 g., m.p. 150–153°) were obtained.

Anal. Found: C, 60.98; H, 8.07; OCH_3, 12.17; C-methyl, 5.11; active H, 1.8. Calcd. for $C_{13}H_{20}O_5$: C, 60.92; H, 7.89; OCH_3, 12.11; C-methyl, 5.87.

This compound shows a peak at 223 mμ with ε 16,100. Reaction with thiosemicarbazide gave ε 21,300 at 291 mμ (calculated from the solution), which is characteristic of α,β-unsaturated aldehydes. It was oxidized by ammoniacal silver but the expected acid could not be isolated.

About one-third of the chloroform distillate was treated with 1.5 g. of dinitrophenylhydrazine and 2 ml. of 5 *N* HCl in 50 ml. of alcohol. The mixture was heated in a glass-stoppered flask and the unreacted dinitrophenylhydrazine filtered from the hot solution. On cooling, more crystals separated. These were extracted with benzene and the combined solutions evaporated to yield 0.48 g. melting at 106–122°. These were chromatographed to yield 0.32 g. of acetone dinitrophenylhydrazone, m.p. 123–125°.

Anal. Found: C, 45.61; H, 3.99; N, 23.61. Calcd. for $C_9H_{10}N_9O_4$: C, 45.38; H, 4.23; N, 23.52.

Identification was confirmed by infrared spectra. Paper chromatography indicated the presence of another dinitrophenylhydrazone in the mother liquors which could not be crystallized or separated completely from acetone dinitrophenylhydrazone.

Ozonolysis of α,β-Unsaturated Aldehyde.—The unsaturated aldehyde (200 mg.) was dissolved in 25 ml. of chloroform and ozonized oxygen bubbled through the solution for 45 minutes. The solution was aerated a few minutes and warmed with a total of about 0.5 g. of Raney nickel to decompose the ozonides. The nickel was removed and the solution extracted three times with water and evaporated to dryness; wt. 142 mg. This was dissolved in alcohol and a portion (46 mg.) was treated with 40 mg. of dinitrophenylhydrazine and 0.2 ml. of concentrated HCl and boiled. Eight mg. of crystals separated which melted above 230°.

Anal. Found: C, 41.12; H, 2.55; N, 27.92. Calcd. for $C_{14}H_{10}N_8O_8$: C, 40.20; H, 2.41; N, 26.79.

The infrared spectrum of a Nujol mull was identical to that of a sample prepared from glyoxal.

A portion of the above alcohol solution and the aqueous extract of the chloroform were separately treated with thiosemicarbazide. The ultraviolet spectrum of each confirmed the presence of a thiosemicarbazone of an aldehyde or ketone (peak at 270 mμ) and glyoxal (peak at 345 mμ) but not of the α,β-unsaturated aldehyde (absence of a peak at 291 mμ).

Dihydroalcohol.—Fumagillin (18.3 g.) was partially dissolved in 250 ml. of alcohol by warming, and hydrogenated at 20 p.s.i. in the presence of 0.183 g. of platinum oxide catalyst. Five moles of hydrogen was absorbed in less than one hour. Most of the alcohol was removed by evaporation after removal of the catalyst, and the residue dissolved in 800 ml. of 0.13 *N* NaOH and kept at 70–75° for 45 min. The solution was cooled and acidified to *p*H 2–3 to precipitate the sebacic acid, which was removed by filtration. The filtrate was adjusted to *p*H 11 with 5 *N* NaOH and extracted continuously with ether for two days. It was found later that one extraction with an equal volume would extract over 90%. The ether extract was evaporated to yield an oil (8.4 g.).

Anal. Found: C, 66.02; H, 10.64; O, 22.89; $[\alpha]_D$, −42° (in ethanol). Calcd. for $C_{16}H_{28}O_4$: C, 67.57; H, 9.92; O, 22.50. For $C_{16}H_{29}O_{4.5}$: C, 65.50; H, 9.96; O, 24.54.

This corresponds fairly well by optical rotation and infrared spectrum to the dihydroalcohol II described by Tarbell.[5] We also have obtained substantially the same compound by hydrogenation of the crude saponification product (alcohol I) from fumagillin: Alcohol I (7.54 g.) was dissolved in 75 ml. of absolute alcohol and hydrogenated at 35–40 p.s.i. One mole of hydrogen was absorbed in less than 1 hr. The alcohol was evaporated to yield an oil (7.36 g.).

Anal. Found: C, 65.70; H, 9.75; active H, 1.4.

Tarbell[5] has obtained the tetrahydroalcohol instead of the dihydroalcohol by hydrogenation of alcohol I which was presumably much more pure than our alcohol I, which is simply the neutral fraction after saponification of fumagillin under mild conditions. Whether one or two moles of hydrogen are absorbed by alcohol I possibly depends on its purity as well as the activity of the catalyst, although we have not investigated this.

Dihydroalcohol prepared by either of the above methods yields the chlorodinitrobenzoate on treatment with dinitrobenzoyl chloride.

Chlorodinitrobenzoate of the Dihydroalcohol.—The dihydroalcohol (7.2 g.) was dissolved in 300 ml. of benzene and about one-third of the solvent distilled to remove traces of water. The solution was cooled and 2.5 ml. of pyridine and 6.0 g. of dinitrobenzoyl chloride added and the mixture boiled for 2 hours. The cooled solution was filtered and washed successively with 5% Na_2CO_3 and dilute HCl. The benzene was evaporated, leaving 12.7 g. of a thick orange oil. Ten ml. of methanol was added and crystals began to form. These were filtered and washed with cold methanol, yielding 4.1 g. (m.p. 125–128°) which was recrystallized from 50 ml. of hot methanol yielding 3.15 g. (m.p. 129–134°). The mother liquors yielded about 2 g. more of crystals, but most of the fractions were oils. Recrystallized for analysis (m.p. 133–135°).

Anal. Found: C, 53.80; H, 5.95; N, 5.52; O, 28.08; Cl, 6.88; OCH_3, 5.9; C-methyl, 6.09, 5.40; active H, 2.09 (CH_3MgI); $[\alpha]_D$ −80.3° (*c* 1% in $CHCl_3$). Calcd. for $C_{28}H_{31}O_9N_2Cl$: C, 53.64; H, 6.07; O, 27.96; N, 5.44; Cl, 6.89; OCH_3, 6.03; C-methyl (2), 5.83.

The chlorine does not react with boiling alcoholic silver nitrate.

Peroxide Oxidation of the Chlorodinitrobenzoate.—Four hundred mg. of the chlorodinitrobenzoate was dissolved in 15 ml. of glacial acetic acid, 1.5 ml. of 30% H_2O_2 added and the solution kept at 70° for 17 hours. The pale yellow solution, still containing peroxide, was evaporated several times with benzene and with methanol. The oil (372 mg.) was washed with water and dissolved in 1:1 benzene–carbon tetrachloride, and passed over an alumina column. A colorless oil (390 mg.) was obtained which showed carbonyl absorption at 5.62 (weak) 5.75 (ester) and 5.86 μ (weak), but weak OH absorption. The infrared spectrum also proved the absence of any unchanged chlorodinitrobenzoate.

Anal. Found: C, 51.97; H, 5.28; N, 5.64; O, 28.84. Calcd. for $C_{21}H_{25}O_9N_2Cl$: C, 52.02; H, 5.20; N, 5.79; O, 29.70.

Periodate Treatment of Chlorodinitrobenzoate.—The chlorodinitrobenzoate (0.1 g.) was dissolved in 5 ml. of methanol and mixed with 0.15 g. of H_5IO_6 in water. Some precipitate formed. The mixture was stirred for 3 days at room temperature and the solid (22 mg.) removed. An aldehyde odor was noted in the solution, so it was steam distilled, but no dinitrophenylhydrazone was obtained from the distillate. The residue (oil and water) was extracted with ether to yield 72 mg. of oil which was dissolved in 1:1 cyclohexane–benzene and chromatographed on HCl-washed alumina. An oil (58 mg.) was obtained which was shown by infrared analysis to differ from the starting chlorodinitrobenzoate although the elementary analyses are substantially the same. This product also shows much weaker –OH-absorption.

Anal. Found: C, 53.78; H, 6.24; N, 5.53.

Apparently no oxidation took place, and possibly only isomerization due to the long contact with acid.

Oxidation of Dihydroalcohol.—Dihydroalcohol (1.2 g.) was suspended in 50 ml. of water and stirred mechanically

and 3.48 g. of $KMnO_4$ added during 3 days. The MnO_2 was filtered and the alkaline solution was extracted with ether to yield about 0.35 g. of unchanged dihydroalcohol. The aqueous solution was acidified and again extracted with ether to yield about 0.2 g. Extraction with butanol yielded about 0.4 g. The ether-soluble material was extracted with petroleum ether to remove a small amount of (probably) isocaproic acid. The rest was dissolved in acetone to separate from some inorganic salts and cyclohexane added. On standing several days, crystals and oil separated. The oil was removed and the crystals (about 9 mg., m.p. 175–183°) washed with 1:1 cyclohexane–acetone.

Anal. Found: C, 42 41; H, 6.78; equiv. wt. by titration, 61. Calcd. for $C_4H_6O_4$: C, 40.68; H, 5.12; equiv. wt., 59.

The infrared spectrum confirmed its identity with succinic acid. From other experiments as much as 0.16 g. of succinic acid has been isolated from the acidic ether extract (1.4 g.) from 11.6 g. of the dihydroalcohol.

From a similar experiment the butanol extract was evaporated and the *p*-bromophenacyl ester prepared in the usual manner; m.p. 214–216°.

Anal. Calcd. for $C_{20}H_{16}Br_2O_6$: C, 46.90; H, 3.15; Br, 31.21. Found: C, 46.47; H, 3.25; Br, 30.37.

Infrared comparison with the *p*-bromophenacyl ester prepared from succinic acid showed them to be identical. The *p*-bromophenacyl ester of succinic acid was much less soluble in alcohol than other *p*-bromophenacyl esters which made it easy to isolate, although the amount present was small. The other *p*-bromophenacyl esters were not isolated in pure form, in spite of many attempts to separate and purify them by chromatography.

Hexahydroalcohol.—Fumagillin (9.2 g.) was dissolved in 200 ml. of glacial acetic acid and hydrogenated in the presence of 4.6 g. of platinum oxide catalyst for 16 hr. Seven moles of hydrogen was absorbed. The acetic acid was removed by evaporation and toluene added and evaporated to more completely remove the acetic acid. The oil was suspended in 112 ml. of water plus 28 ml. of 5 *N* NaOH and the mixture boiled for 80 min. After cooling, the separated oil was extracted 3 times with ether. The ether was removed to leave an oil.

Anal. Found: C, 66.84; H, 11.26; C-methyl, 9.79; active H, 2.5; $[\alpha]D$, −28° (*c* 4% in ethanol). Calcd. for $C_{16}H_{32}O_4$: C, 66.63; H, 11.18; C-methyl (2), 10.42.

Chromatography of similar preparations on alumina showed that they were frequently contaminated with some tetrahydroalcohol which could be crystallized from the eluates (1% methanol in benzene) which preceded the hexahydroalcohol. Furthermore, minor variations in the infrared spectra of various hexahydroalcohol preparations convinces us that none of the preparations may be considered pure. Hydrogenation of the crystalline tetrahydroalcohol with 1 mole of hydrogen (see below) yields a product with substantially the same infrared spectrum as the above hexahydroalcohol.

Hydrogenation of Tetrahydroalcohol I.—Crystalline tetrahydroalcohol I (1 g.) was dissolved in 50 ml. of glacial acetic acid and hydrogenated with 0.5 g. of platinum oxide catalyst for 17 hours. The solution was filtered, evaporated to an oil and taken up in 2:1 benzene–petroleum ether for chromatography on 18 g. of alumina. The main fraction (0.69 g.) was eluted with 2% methanol in benzene.

Anal. Found: C, 67.3; H, 11.3; OCH_3, 9.8; active H, 2.9. Calcd. for $C_{16}H_{32}O_4$: C, 66.63; H, 11.18; OCH_3, 10.76.

The infrared spectrum was substantially the same as for preparations of the hexahydroalcohol prepared by hydrogenation of fumagillin followed by saponification. In a small scale experiment (37 mg.) the uptake of hydrogen was 1.09 moles per mole of tetrahydroalcohol.

Bis-dinitrobenzoate of the Hexahydroalcohol.—Fumagillin (4.58 g.) was dissolved in 100 ml. of glacial acetic acid and hydrogenated in the presence of 2 g. of platinum oxide catalyst. Approximately 7 moles of hydrogen was absorbed.

The acetic acid was removed by evaporation and the oil dissolved in 56 ml. of water plus 14 ml. of 5 *N* NaOH and refluxed for 75 min. The solution was cooled and extracted with ether. The ether was evaporated to give 3 g. of an oil (the hexahydroalcohol) which was dissolved in 30 ml. of dry pyridine and refluxed with 5 g. of 3,5-dinitrobenzoyl chloride for 20 min. The pyridine was removed by evaporation and the residue dissolved in 100 ml. of ether. The ether was washed 3 times with 5% sodium carbonate solution and twice with water. Evaporation of the ether yielded 6.4 g. of an oil.

On standing overnight with methanol, 2.3 g. of crystals melting at 128–135° separated. These were dissolved in benzene and passed over an alumina column. Fractions eluted with 1 and 2% methanol in benzene yielded 1.1 g. of crystals when evaporated to dryness and treated with methanol; m.p. 167–169°. A different preparation gave 4.82% methoxyl (calcd. 4.58). Infrared analysis (Nujol mull) showed no evidence of hydroxyl groups.

Anal. Found: C, 53.17; H, 5.30; N, 8.44. Calcd. for $C_{30}H_{36}N_4O_{14}$: C, 53.25; H, 5.36; N, 8.28.

Dehydrogenation of Hexahydroalcohol.—A hexahydroalcohol preparation (3.8 g.) which was not homogeneous, according to its infrared spectrum, was mixed with 8 g. of selenium and heated at 320° with collection of the volatile product in a cup under a cold finger. About 380 mg. of "water" and about 500 mg. of a pale yellow thin oil and some selenium metal (?) were collected in the cup during 17 hours. The water was separated and heated to 100°, but no methanol was recovered.[11] Possibly it escaped, since none of the products contain methoxyl. The oil was distilled at a bath temperature of 200–250°. Three fractions totaling 303 mg. were obtained. They were quite similar.

Anal. Fraction 1: C, 74.7; H, 12.2.

The infrared spectrum showed presence of carbonyl, hydroxyl and carbon–carbon double bond. Reaction with thiosemicarbazide indicated presence of a non-conjugated carbonyl.[12]

On the basis of the above information, the remainder (250 mg.) was treated with 200 mg. of dinitrophenylhydrazine in the usual manner and 377 mg. of a dinitrophenylhydrazone obtained as an oil. Chromatography on acid-washed alumina yielded 304 mg. of a dinitrophenylhydrazone melting at 40–45° plus two minor components. The crystals were rechromatographed on alumina and again on a reversed phase chromatogram.[13] Several fractions were combined and recrystallized from alcohol to yield 10 mg., m.p. 59–64°.

Anal. Found: C, 54.62; H, 6.41; N, 18.26. Calcd. for $C_{14}H_{20}N_4O_4$: C, 54.53; H, 6.54; N, 18.17.

The other fractions were not crystallized and contained higher oxygen content, suggesting that some ketones containing more oxygen were also formed during the dehydrogenation. The hydroxyl band in the infrared spectrum of the crude distillate confirmed this.

For comparison, ethyl isoamyl ketone was prepared by reaction of isocapronitrile with ethylmagnesium iodide in the usual manner. The crude product was treated with dinitrophenylhydrazine and the dinitrophenylhydrazone chromatographed on acid-washed alumina and the main fractions crystallized from ethanol, m.p. 59–64°.

Anal. Found: C, 54.72; H, 6.26; N, 18.44, 18.64.

The infrared spectrum of a Nujol mull was identical with the spectrum of the sample obtained from the selenium dehydrogenation.

The non-volatile products of the dehydrogenation were separated from the selenium by dissolving in acetone. About 0.5 g. of a creosote-like material was obtained. This was dissolved in 1:1 petroleum ether–benzene and passed over Merck reagent alumina and the early fractions combined to give 0.3 g. of a yellow oil.

Anal. Found: C, 82.22; H, 9.74; O, 7.64; OCH_3, trace.

The infrared curve showed carbonyl (5.84 μ) and aromatic ring (6.21, 6.30, 6.72 μ) but no –OH.

This was passed over another alumina (20 g.) column. The early fractions (petroleum ether) gave 97 mg. of a colorless pungent oil and later fractions (1:1 petroleum ether–benzene) yielded 103 mg. of a red oil.

The first fraction showed peaks in the ultraviolet at 255 mμ (*a* 54.3) and at 285 mμ (*a* 14.3). It shows bands at 6.13,

(11) Tarbell[5] was able to isolate methanol at this point from dehydrogenation of the tetrahydroalcohol.

(12) L. K. Evans and A. E. Gillam, *J. Chem. Soc.*, 565 (1943).

(13) G. A. Howard and A. R. Tatchell, *Chemistry & Industry*, 219 (1954).

6.21 and 6.30 μ in the double bond region. Two model benzofurans showed no band at 6.13.

Anal. Found: C, 84.03; H, 9.36. Calcd. for $C_{15}H_{20}O$: C, 83.28; H, 9.32. For $C_{15}H_{18}O$: C, 84.07; H, 8.47.

The second fraction showed only end absorption in the ultraviolet (down to 220 mμ). A similar one from Pd-charcoal dehydrogenation showed a peak at 218 mμ (a 38.0). The infrared spectrum showed carbonyl absorption (5.84 μ) and double bond absorption (6.24 and 6.31 μ). Benzaldehyde also shows these three peaks.

Anal. Found: C, 81.74; H, 10.05. Calcd. for $C_{18}H_{18}O$: C, 82.06; H, 9.54. For $C_{13}H_{20}O$: C, 81.20; H, 10.48.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF ROCHESTER]

The Chemistry of Fumagillin.[1a] III

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The alcohol moiety of fumagillin, alcohol-I ($C_{16}H_{26}O_4$), contains a secondary alcoholic hydroxyl group, a methoxyl group, and two non-carbonyl oxygen functions; it shows no absorption in the ultraviolet. Catalytic reduction of alcohol-I yields the crystalline tetrahydroalcohol-I, which contains two hydroxyl groups, one secondary and one tertiary. Permanganate oxidation of tetrahydroalcohol-I gives isocaproic acid; treatment with mineral acid isomerizes tetrahydroalcohol-I to carbonyl-containing products, and oxidation with chromic oxide–pyridine yields a saturated ketone. Numerous other transformations of fumagillin derivatives are described.

Earlier work[4-6] has shown that fumagillin has the empirical formula $C_{26}H_{34}O_7$, and this has been confirmed by an X-ray crystallographic study.[7] Alkaline hydrolysis of fumagillin yields decatetraenedioic acid and a neutral substance, $C_{16}H_{26}O_4$, which we have named alcohol-I.

This material is a viscous high boiling oil, which has not been obtained crystalline, and which, in spite of varied and sustained efforts, has not yielded any crystalline derivatives. It contains a secondary hydroxyl group, a methoxyl group, and two non-carbonyl oxygen atoms; it shows no absorption in the carbonyl region of the infrared, and no absorption in the ultraviolet. The carbon–oxygen linkages are subject to hydrogenolysis, and alcohol-I consumes one equivalent (*i.e.*, 0.25 mole) of lithium aluminum hydride, in addition to the equivalent which reacts with the hydroxyl group.

Treatment of alcohol-I with hydrogen and platinum catalyst in alcohol gives an uptake of almost two moles of hydrogen, and chromatography on alumina of the resulting products leads to isolation of the crystalline tetrahydroalcohol-I, $C_{16}H_{30}O_4$, m.p. 89–90°. The success of this reduction depends both on the quality of the alcohol-I and of the platinum catalyst. In the best cases, the yield of crystalline material reaches 70%, but in other runs, only slightly more than one mole of hydrogen is absorbed, and a viscous dihydroalcohol-I (see below) is obtained, with only traces of the crystalline tetrahydro compound.

The tetrahydroalcohol-I results from the addition of one mole of hydrogen to a carbon–carbon double bond, and from the hydrogenolysis of a carbon–oxygen linkage by the second mole of hydrogen. This is demonstrated by the presence of two active hydrogens in the compound, by the fact that, in contrast to alcohol-I, it is unaffected by lithium aluminum hydride, and by the oxidation to a hydroxyketone (below).

Oxidation of tetrahydroalcohol-I by permanganate in 10% sulfuric acid yielded isocaproic acid, which was identified (through the crystalline *p*-bromophenacyl ester) by mixed m.p. and by the correspondence of the infrared spectra with an authentic sample. This evidence shows the presence of the $>CCH_2CH_2CH(CH_3)_2$ grouping in the molecule, and, coupled with the isolation of acetone from ozonization,[6] indicates the presence of the $>CCH_2$-$CH{=}C(CH_3)_2$ group in alcohol-I and in fumagillin

The other product from the acid permanganate oxidation of tetrahydroalcohol-I was a neutral compound, showing strong absorption at 1706 cm.$^{-1}$, and of composition agreeing with the formula C_{16}-$H_{30}O_4$; it apparently was a ketonic isomerization product produced by action of the sulfuric acid on tetrahydroalcohol-I. Treatment of tetrahydroalcohol-I with aqueous alcoholic hydrogen chloride, or with Dowex-50, gave materials with strong absorption in the 1720–1706 cm.$^{-1}$ region; the hydrochloric acid appeared to introduce some chlorine into the molecule, and the product showed absorption in the ultraviolet[8]: 227 (3.4), 278 (3.8).

Treatment of tetrahydroalcohol-I with the elegant chromic oxide–pyridine reagent,[9] which, being

(1a) Part of this material was presented before the Division of Medicinal Chemistry at the American Chemical Society Meeting in New York, September, 1954; D. S. Tarbell, H. R. Al-Kazimi, P. Hoffman, G. A. Page, H. R. Vogt, J. R. Schenck, M. P. Hargie and A. Isarasena, Abstracts of New York Meeting, 1954, p. 17 N.

(1b) Abbott Laboratories Fellow, 1952–1953.

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(3) Abbott Laboratories Fellow, 1953–1954.

(4) T. E. Eble and F. R. Hanson, *Antibiotics and Chemotherapy*, **1**, 54 (1951).

(5) J. R. Schenck, M. P. Hargie, D. S. Tarbell and P. Hoffman, THIS JOURNAL, **75**, 2274 (1953).

(6) J. R. Schenck, M. P. Hargie and A. Isarasena, *ibid.*, **77**, 5606 (1955).

(7) C. J. Brown and J. K. Landquist, *Chemistry & Industry*, 973 (1953).

(8) Ultraviolet spectra are indicated throughout by giving λ_{max} in mμ, and log ϵ in parentheses.

(9) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 425 (1953).