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CONSTITUTION AND STEREOCHEMISTRY OF ENMEIN, A DITERPENE FROM *ISODON TRICHOCARPUS* KUDO

T. KUBOTA, T. MATSUURA and T. TSUTSUI
Faculty of Science, Osaka City University, Sugimoto-cho, Sumiyoshi-ku, Osaka, Japan

and

S. UYEO, H. IRIE, A. NUMATA, T. FUJITA and T. SUZUKI
Faculty of Pharmaceutical Sciences, Kyoto University, Yoshida, Sakyo-ku, Kyoto, Japan

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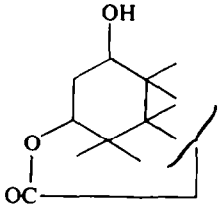
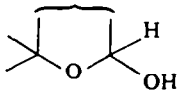
Abstract—The total structure of enmein, a diterpene, isolated from *Isodon trichocarpus* Kudo has been established as shown in the formula If.

Isodon trichocarpus KUDO (Japanese name, Enmei-sô), a plant of the Labiatae family, has long been used as a home remedy for gastrointestinal troubles. Its bitter principle, enmein[1,2] (= isodonin[3]), was isolated a few years ago by three groups of researchers. It is a diterpene with a molecular formula of $C_{20}H_{26}O_6$.[3] Its chemical structure was studied by many workers[1–8] and six oxygen atoms in enmein were found to be distributed in three partial structures.

(i) Three oxygens are present in structure Ia,[8] two as a δ-lactone[1–3] and one as a hydroxyl group.
(ii) Two oxygens are in structure Ib,[8] as a cyclic hemiacetal.
(iii) The remaining one oxygen is present in structure Ic,[4,8] as an α,β-unsaturated cyclopentanone.



Ia



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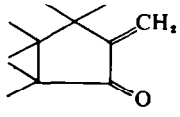
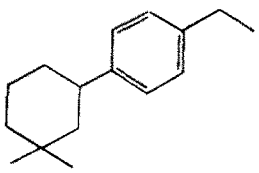


Ic

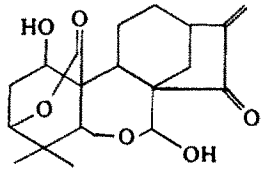
Kanatomo obtained 1-ethyl-4-(3,3-dimethylcyclohexyl) benzene (II) by the baryta

[1] T. Ikeda and S. Kanatomo, *Yakugaku Zasshi* **78**, 1123 (1958).
[2] M. Takahashi, T. Fujita and Y. Koyana, *Yakugaku Zasshi* **78**, 699 (1958).
[3] K. Naya, *Nippon Kagaku Zasshi* **79**, 885 (1958).
[4] M. Takahashi, T. Fujita and Y. Koyama, *Yakugaku Zasshi* **80**, 594, 696 (1960).
[5] T. Ikeda, T. Kosuge and S. Kanatomo, *Yakugaku Zasshi* **78**, 947 (1958).
[6] S. Kanatomo, *Chem. Pharm. Bull. Tokyo* **6**, 680 (1958).
[7] S. Kanatomo, *Yakugaku Zasshi* **81**, 1049 1437 (1961).
[8] T. Kubota, T. Matsuura, T. Tsutsui and K. Naya, *Bull. Chem. Soc. Japan* **34**, 1737 (1961); *Nippon Kagaku Zasshi* **84**, 353 (1963). A large part of this work was read as a paper at the 5th Symposium on the Chemistry of Natiral Organic Compounds at Sendai (1961).

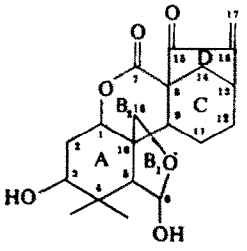
dry distillation of enmein, and retene from selenium dehydrogenation of a reduction product of dihydroenmein with LAH.[7] Based on these experimental results, Kubota and others proposed the structural formula Id for enmein.[8]



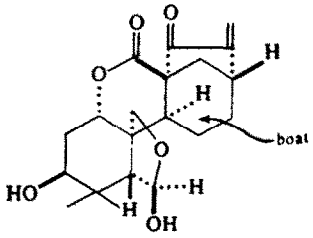
II



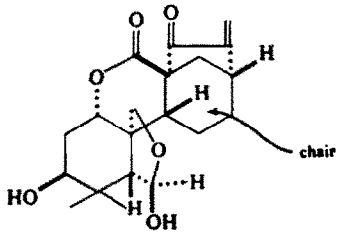
Id



Ie



If



Ig

Later, this work was taken up as co-operative study* and the plane structure of enmein was corrected to formula Ie,† with stereostructure presumed to be either formula If or Ig.[8,9] At the same time, Iitaka and Natsume[10] revealed that enmein has the structure represented by If or its antipode from the result of X-ray crystallographic analysis of the monobromoacetate of dihydroenmein 3-monoacetate. Consequently, the total structure of enmein would be represented as If.

The present paper reports the results of work carried out on this compound.‡

(1) *Structure of ring* A

The partial structure of Ia for enmein was derived from the following series of reactions.[8] Mild reduction with sodium borohydride gives dihydroenmein[1-3,§] (III),

* Three groups of researchers worked in six universities, Osaka City University, Kyoto University, Kyoto Prefectural Medical College, Kanazawa University, Tokyo University and Shizuoka College of Pharmacy.

† The numbering of carbon atoms in enmein has been given as in formula (Ie), assuming its derivation from the diterpene kaurene and phyllocladene. Rings are designed similarly as A, B_1, B_2, C and D.

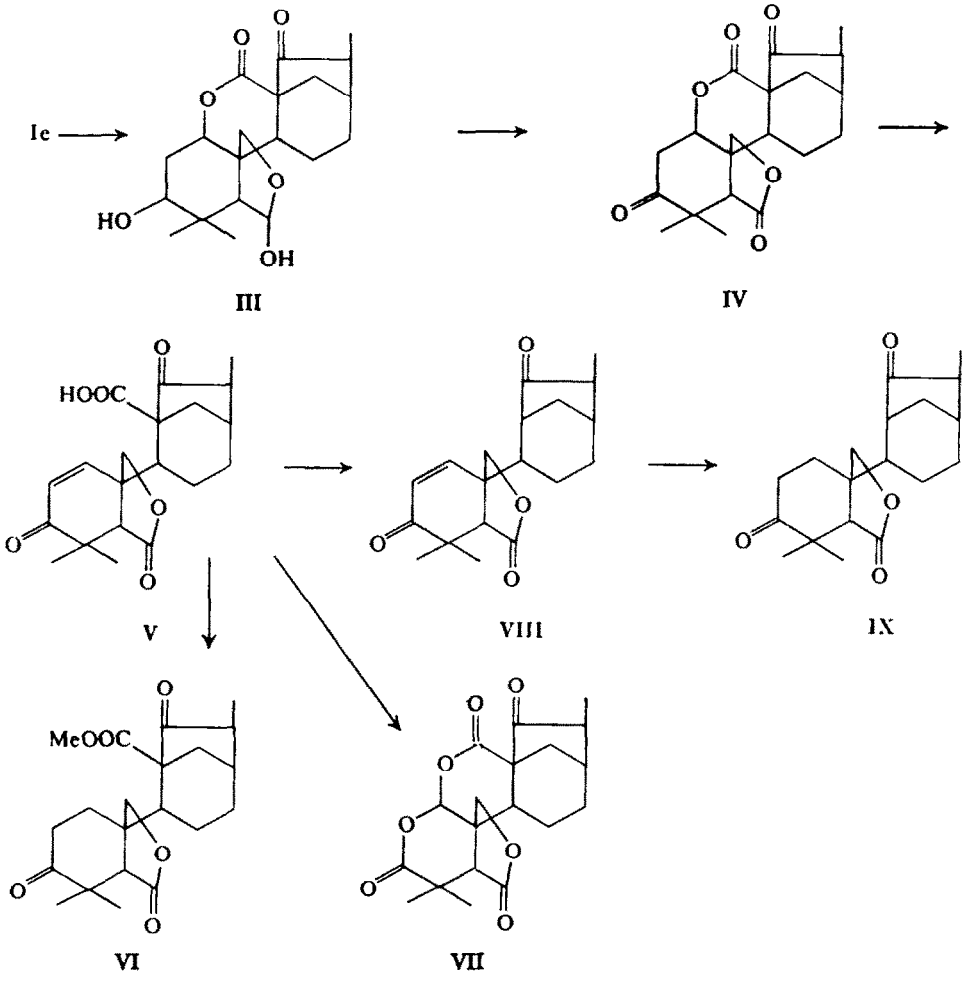
‡ At the Osaka City University and Kyoto University.

§ β-Dihydroenmein, which was obtained together with α-dihydroenmein by the catalytic hydrogenation of enmein in acetic acid, has been identified as α-dihydroenmein 6-acetate. Therefore the name of α-dihydroenmein designated in the previous reports[8,9] has been renamed simply dihydroenmein.

[9] T. Kubota, T. Matsuura, T. Tsutsui, S. Uyeo, M. Takahashi, H. Irie, A. Numata, T. Fujita, T. Okamoto, M. Natsume, Y. Kawazoe, K. Sudo, T. Ikeda, M. Tomoeda, S. Kanatomo, T. Kosuge and K. Adachi, *Tetrahedron Letters* 1243 (1964).

[10] Y. Iitaka and M. Natsume, *Tetrahedron Letters* 1257 (1964).

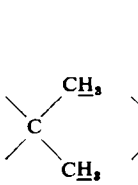
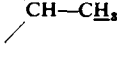
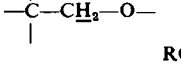
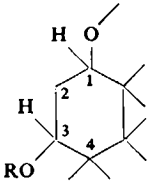
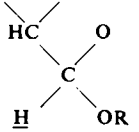
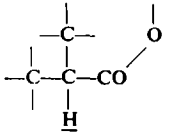
which on oxidation with chromium trioxide–acetic acid affords bisdehydrodihydroenmein (IV).[3] Hydrolysis of IV under a mild condition (N/60 alkali) results in consumption of 1 mole of alkali to form an isomeric acid (V), λ_{max} 226 mμ. The NMR spectrum (Table 1) of the methyl ester of V indicates an AB-type quartet for the two protons of the double bond and agrees with the structure of V derived from the partial structure Ia. Catalytic reduction of the methyl ester of V gives a dihydro compound (VI), while ozone oxidation of V gives a nor compound (VII). Under mild conditions, VII consumes 2 moles of alkali and is positive to aldehyde reagents. Pyrolysis of V results in decarboxylation and the compound VIII, λ_{max} 225 mμ, is formed. Catalytic reduction of VIII gives a dihydro compound (IX). These compounds proved to be key intermediates in the structural determination of enmein and each of these structures may be indicated on the basis of the plane structure Ie.



(2) *Structure of rings* C *and* D

The presence of α-methylenecyclopentanone system (Ic) in enmein has already been reported.[2,4,8] Kanatomo assumed a phyllocladene structure for enmein from the

TABLE 1. NMR SPECTRA*

Compounds \ Types of Protons								Other Protons
IV[a]	8·61 (s) 8·56 (s)	8·97 (d) (J = 6·5)	5·5 (d) 4·97 (d) (J = 10)	4·75 (q)[a] (J_{AX} = 11·8) (J_{BX} = 6·3)		7·16 (s)		C_2-protons H_A 7·0 (q) H_B 6·42 (q) (J_{AB} = 14·1) (J_{AX} = 11·8) (J_{BX} = 6·3)
Methyl Ester of V	8·60 (s) 8·71 (s)	8·87 (d) (J = 7)	5·80 (s)			6·80 (s)	3·24 (d) 3·92 (d) (J = 11·4)	—COOMe 6·22 (s)
VI	8·84 (s) 8·72 (s)	8·9 (d) (J = 7)	5·34 (d) 5·97 (d) (J = 12)			7·37 (s)		—COOMe 6·30 (s)
VIII	8·77 (s) 8·59 (s)	8·8 (d) (J = 7)	5·53 (d) 5·81 (d) (J = 10)				3·48 (d) 3·82 (d) (J = 10)	
XXVI	8·57 (s) 8·83 (s)	8·90 (d) (J = 8)	5·62 (d) 5·87 (d) (J = 11·4)			7·22 (s)	3·63 (d) 4·08 (d) (J = 11·4)	—COOMe 6·32 (s) 6·47 (s)
XXIX	8·71 (s) 9·03 (s)	8·91 (d) (J = 7)	5·79 (d) 6·05 (d) (J = 10)	4·76 (q)[b] (J_{AX} = 2·5) (J_{BX} = 1·5)		7·53 (s)	4·55 (t) broad	—COOMe 6·32 (s) 6·37 (s) —OAc 7·87 (s)
XXX	8·94 (s)	8·86 (d) (J = 9)	5·94 (s)	5·11 (t?) 5·34 (t?)	3·84 (s)			—OAc 7·89 (s) 7·98 (s)
XXXI	9·00 (s)	8·88 (d) (J = 6·5)	6·02 (s)	5·15 (m) 5·40 (m)	4·62 (d) (J = 2)			—OAc 7·90 (s) 7·11 (s)
XXXII	8·83, 8·87	8·80 (d) (J = 7)	5·66 (d) 5·92 (d)	4·99 (t)[b] (J_{AX} = J_{BX} = 2·7) 5·35 (q)[a] (J_{AX} = 7) (J_{BX} = 11)		7·50 (s)		—OAc 7·88 (s)

XLI	8·67 (s) 8·71 (s)	8·89 (d) (J = 7)	5·75 (d) 6·08 (d) (J = 10)			7·44 (s)	—COOMe 6·32 (s)
XLIV	7·73 (s)[c] 8·05 (s)[c]	8·70 (d) (J = 7)	5·67 (d) 6·28 (d) (J = 12)				—COOMe 6·35 (s) (6H) 6·46 (s) (3H)
XLV[f]	8·83 (s)	8·77 (d) (J = 8)	5·86 (d) 6·20 (d) (J = 9)	4·93 (m)	5·28 (d)		—OMe 6·68 (s)
LIV	8·78 (s) 8·94 (s)	9·00 (d) (J = 6·5)	5·67 (d) 6·23 (d) (J = 10)	5·55 (q)[a] (J_{AX} = 6·5) (J_{BX} = 10·5)		7·68 (s)	
Methyl Ester of LIX	8·80 (s) 8·93 (s)	8·86 (d) (J = 8)	5·55 (d) 6·25 (d) (J = 9·5)	5·69 (t)[a] (J_{AX} = J_{BX} = 8)		7·70 (s)	
LVII	8·83 (s) 8·98 (s)	9·00 (d) (J = 6)	5·71 (d) 6·05 (d) (J = 10)			7·89 (s)	
LXVII	8·94 (s)	8·80[d]	5·93 (s)	5·10 (t?) 5·40 (m)	3·85 (s)		$=CH_2$ 3·92 (s) 4·47 (s)
LXX[e]	8·66 (s) 8·56 (s)	8·95 (d) (J = 6)	5·49 (d) 5·06 (d) (J = 10)	4·73 (q)[a] (J_{AX} = 2·5) (J_{BX} = 3·7)		7·00 (s)	C_2-protons H_A 7·11 (q) H_B 6·40 (q) (J_{AB} = 15·7) (J_{AX} = 2·5 (J_{BX} = 3·7)
LXXIII	8·90 (s) 8·81 (s)	8·85 (d) (J = 5·5)	5·92 (d) 5·65 (d) (J = 10)	5·43 (q)[a,b] (2H) (J_{AX} = 5·6) (J_{BX} = 11·5)		7·70 (s)	—OAc 7·87 (s)

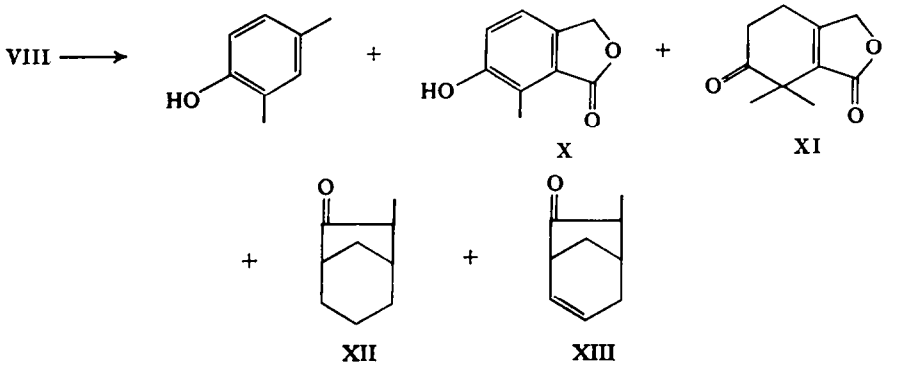
[*] τ-Values; tetramethylsilane internal standard; J = c/s.

[a] C_1-Proton.
[b] C_2-Proton.
[c] Isopropylidene methyl protons.
[d] Methyl protons of dihydroenmein diacetate contaminated.
[e] Taken in pyridine.
[f] Determined at 56·4 Mc.

results of the afore-mentioned selenium dehydrogenation and baryta distillation, and presumed that this five-membered ring ketone constituted ring D in phyllocladene. Kubota and others also assumed that the rings C and D in enmein were a phyllocladene type, as shown by Id.[8] In order to prove these assumptions, the following experiment was carried out.

The decarboxylated VIII can also be obtained in a good yield directly from bis-dehydrodihydroenmein (IV) by pyrolysis. Pyrolysis of VIII in a low-pressure sealed tube at 350–360° afforded the following six products: Liquid phenol (A); a phenolic lactone (B), $C_9H_8O_3$, m.p. 193–197°; phenol (?; C), m.p. 120–129°; a ketonic lactone (D), $C_{10}H_{12}O_3$, m.p. 95–98°; a mixture of saturated ketone (E) and unsaturated ketone (F).

The liquid phenol (A) was converted into a crystalline 3,5-dinitrobenzoate and confirmed as 2,4-xylenol. The phenolic lactone (B) showed absorptions at 3390 (hydroxyl), 1727 (aromatic γ-lactone), 1615, and 1511 cm^{-1} (aromatic ring) in its IR spectrum, and its NMR spectrum (in pyridine) exhibited a singlet at 7·15 τ for one methyl group bonded to a benzene ring and a singlet at 4·92 τ corresponding to a methylene group in the phthalide.[11] From these spectral data and the formation of 2,4-xylenol, the structure X was assumed. The phenol (C) was obtained in a very minute amount and no further examination was made.



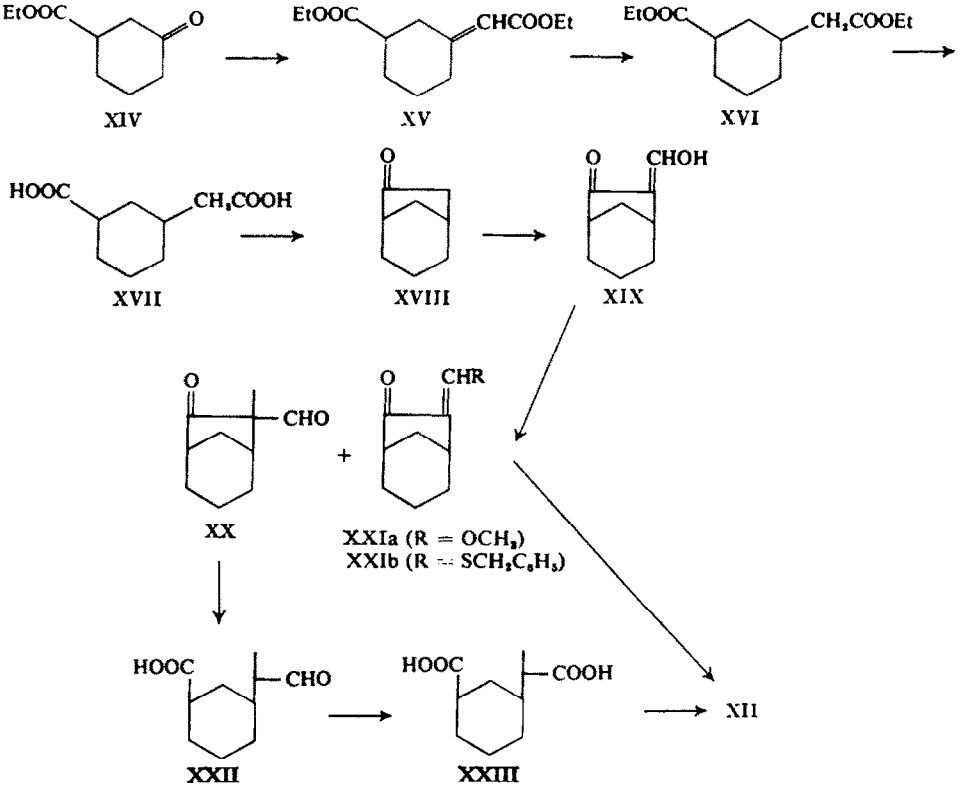
The product D exhibited absorption maximum at 215 mμ (ε 9,600) in its UV and IR spectra showed absorptions at 1753 (α,β-unsaturated γ-lactone), 1719 (6-membered ring ketone), and 1678 (double bond)[12] cm^{-1}. The NMR spectrum of D did not reveal the presence of a vinyl proton so that the double bond must have four substituents. The singlet at 8·59 τ corresponding to 6 protons is thought to be the *gem*-dimethyl group adjacent to the ketone group. The singlet (2 protons) at 5·24 τ may be assigned to the proton of a methylene group bonded to the ring oxygen atom in the lactone and the adjacent carbon atom is not substituted with a hydrogen. These facts and formation of 2,4-xylenol by pyrolysis suggest that the product (D) has a structure shown by XI.

The remaining two products, (E and F), are obtained as a liquid mixture the IR

[11] N. S. Bhacca, D. P. Hollis, L. F. Johnson and E. A. Pier (Varian Associates), *NMR Spectra Catalog* Vol. 2, No. 496 (1963).

[12] E. Schreier, *Helv. Chim. Acta* **46**, 75 (1963).

spectrum of which exhibits absorptions at 1730 and 1633 cm^{-1}, suggesting the presence of a compound having a five-membered ring ketone and a double bond. Conversion of this liquid mixture into 2,4-dinitrophenylhydrazone gives two kinds of crystalline derivatives; $C_{15}H_{18}O_4N_4$, m.p. 180–186°, and $C_{15}H_{16}O_4N_4$, m.p. 136–140°. Catalytic reduction of this liquid mixture gives a liquid product the IR spectrum of which has absorption only at 1730 cm^{-1}, and only one kind of 2,4-dinitrophenylhydrazone, m.p. 180–186°. The NMR spectrum of the semicarbazone, $C_{10}H_{17}ON_3$, of the reduction product shows a doublet (J 6·5 c/s) corresponding to one methyl group. These experimental results and the assumption that enmein has a phyllocladene-type C/D ring suggest the structures XII and XIII, respectively, for the components D and E of the liquid mixture. In order to prove this assumption, synthesis of XII was carried out as shown below.



The compound XIV[13–15] was converted to XV by the Wittig reaction, and XV was catalytically reduced to XVI, which was saponified to obtain the dicarboxylic acid[16] (XVII). The calcium salt of XVII was submitted to pyrolysis in a sealed tube and XVIII was obtained in a high yield. Condensation of XVIII with ethyl formate gave XIX the structure of which was confirmed by spectroscopic data of its semicarbazone.

[13] H. Offermann, *Liebigs Ann.* **280**, 1 (1894).
[14] M. F. Clarke and L. N. Owen, *J. Chem. Soc.* 2108 (1950).
[15] A. Einhorn and F. Coblitz, *Liebigs Ann.* **291**, 297 (1896).
[16] V. N. Ipatieff, J. E. Germain, W. W. Thompson and H. Pines, *J. Org. Chem.* **17**, 272 (1952).

The IR spectrum of this semicarbazone exhibited an absorption at 1647 cm^{-1} for a double bond besides the characteristic absorptions for a semi-carbazone. The NMR spectrum of this semicarbazone showed a signal for the hydrogen atom in one hydroxyl at 5·13 τ, which disappeared on addition of deuterium oxide, and a broad peak at 3·28 τ which may be assigned to —C=C—O—. Consequently, this semicarbazone

may be taken as a monosemicarbazone obtained by the reaction of the ketone group in XIX. Derivation of XII from XIX by direct catalytic reduction did not materialize but was effected by the following two routes. One was the methylation of XIX with methyl iodide, which afforded a mixture of ketoaldehyde (XX) and an enol ether (XXIa), and treatment of this mixture with hydrochloric acid–ethanol resulted in hydrolysis of only XXIa to XIX. Removal of this enolizable XIX left XX which on treatment with alkali to effect deformylation did not give XII but resulted in acid cleavage to form the aldehydic acid (XXII). Oxidation of XXII with nickel peroxide gave the dicarboxylic acid (XXIII) the calcium salt of which gave the expected XII on pyrolysis. The other route from XIX to XII involved desulfuration of the benzyl thioether (XXIb) of XIX with Raney nickel[17] which gave the desired XII in a good yield. The IR spectrum of the semicarbazone of XII in chloroform solution was in complete agreement with that of the semicarbazone of the natural product.

Fission of the C—C bond, such as that in the pyrolysis of VIII, is often used in the elucidation of the carbon skeleton of triterpenoid compounds.[18] Considering the formation of 2,4-xylenol and compounds X to XIII from VIII, and the formation of II by the baryta dry-distillation of enmein, the structure of VIII with α,β-unsaturated ketone group is likely to be represented by formula VIII. At the same time, the presence of 6-methyl-7-oxobicyclo(3.2.1)octane (XII) in dihydroenmein has been proved.

Heating of bisdehydrodihydroenmein (IV) or the acid (V) with hydriodic or hydrochloric acids in acetic acid results in the formation of two isomeric dicarboxylic acids, $C_{20}H_{26}O_7$; XXIV, m.p. 302–303° and XXV, m.p. 270–271°, in approximately 5:1 ratio. Both these acids show UV absorption maxima at 228 mμ and their pK_a values in a Methyl Cellosolve–water (8:2) system agree with the value of an aliphatic dicarboxylic acid of over C_4.[19] The compounds XXIV and XXV are most probably epimeric at 8-position.

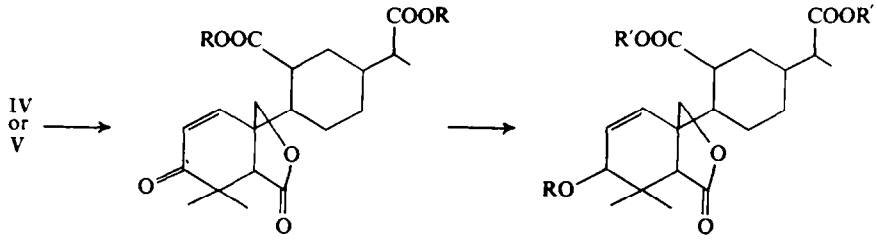
Treatment of bisdehydrodihydroenmein (IV) with sulfuric acid in ethanol gives the ethyl ester of V and hydrolysis of this ethyl ester gives XXIV. These reactions can be explained as the cleavage of the D ring by acid cleavage of the β-ketolactone group in IV or β-ketocarboxylic acid group in V. In fact, the pK_a value of the keto-acid (V) agrees with the values of β-keto acid.[8] However, such evidence alone does not allow the conclusion to be drawn that the lactone group originally present in enmein is bonded to the β-position of the carbonyl group in the D ring of enmein. This point will be taken up in Section (5).

[17] R. E. Ireland and J. A. Marshall, *J. Org. Chem.* **27**, 1615 (1962).

[18] cf. L. Ruzicka, F. Ch. van der Sluys-Veer and S. L. Cohoen, *Helv. Chim. Acta* **22**, 350 (1939); L. Ruzicka, F. Ch. von der Sluys-Veer and O. Jeger, *Ibid.* **26**, 280 (1943).

[19] H. C. Brown, D. H. McDaniel and O. Hoffinger, *Determination of Organic Structures by Physical Method* p. 567. Academic Press, New York (1955).

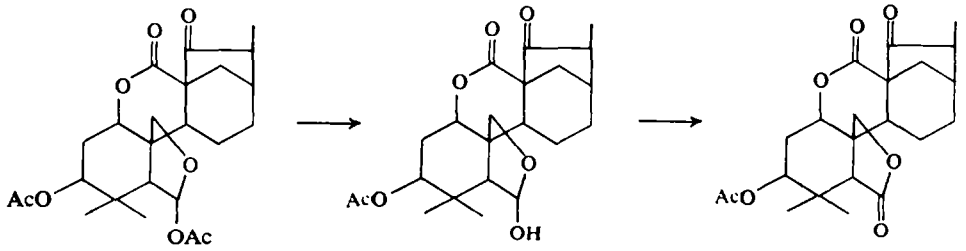
Reduction of the dicarboxylic acid (XXIV) with sodium borohydride gives XXVIII which no longer shows the UV absorption maximum (226 mμ) of an α,β-unsaturated ketone, and gives the dimethyl ester (XXIX) via the monoacetate.

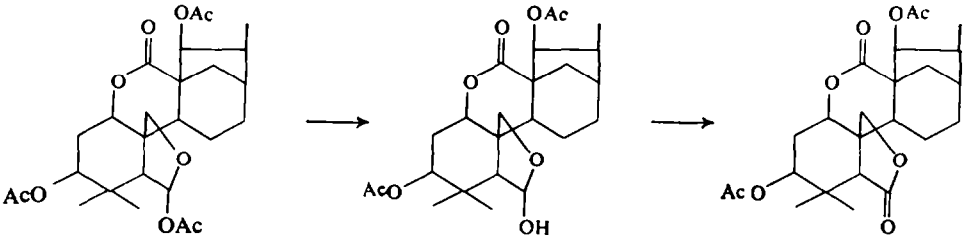


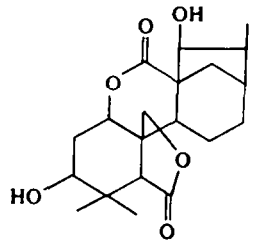
XXIV (R = H) XXV (R = H) XXVIII (R = H, R′ = H)
XXVI (R = Me) XXVII (R = Me) XXIX (R = Ac, R′ = Me)

(3) *Structure of* B_1 *ring and its neighbourhood*

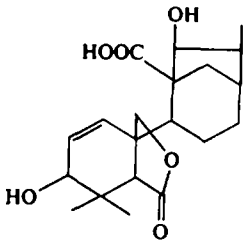
Kubota and others[8] assumed that the hemiacetal ring of enmein is a seven-membered ring, as indicated in formula Id. However, examination of the IR absorption spectra of the lactone ring formed by oxidation of this hemiacetal ring in numerous derivatives of enmein indicate that this ring is a γ-lactone ring. For example, hydrolysis of the diacetate[2,4,8] (XXX) of dihydroenmein and triacetate[2,4,8] (XXXIII) of tetrahydroenmein, in the presence of oxalic acid, gives XXXI and XXXIV, respectively, formed by the hydrolysis of the acetyl group alone in the hemiacetal. Oxidation of XXXI and XXXIV with chromic acid respectively gives the lactones (XXXII and XXXV), which show the absorption for γ-lactone at 1780 cm^{-1} in their IR spectrum.



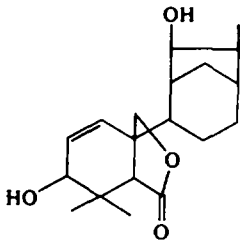




XXXVI



XXXVII



XXXVIII

Besides the above two compounds, a number of enmein derivatives like IV, V, methyl ester of V, VI, VIII and IX show the absorption of γ-lactone at 1770–1790 cm^{-1}. Therefore, the hemiacetal ring (B_1 ring) in enmein must be five-membered.

The diol (XXXVI) obtained by the reduction of bisdehydrodihydroenmein (IV) with sodium borohydride, which incidently reverts to IV by chromic acid oxidation, shows the absorption of γ-lactone at 1766 cm^{-1}.* The two diols (XXXVII and XXXVIII) formed by the borohydride reduction of the acid (V) or its decarboxylated compound VIII, show the γ-lactone absorption at 1745 and 1720 cm^{-1},* while the diacetate of XXXVI shows the absorption at 1780 cm^{-1}.

The NMR spectra (Table 1) of enmein diacetate[3] (LXVII) (3·85 τ) and dihydroenmein diacetate (XXX, 3·84 τ) show a sharp singlet of the proton of —CH(OAc)—O— of the hemiacetal acetate.[20] On the other hand, the NMR spectra of dihydroenmein 3-monoacetate (XXXI; 4·62 τ) and O-methyldehydrodihydroenmein[18] (XLV′; 5·28 τ) exhibit the doublet with a coupling constant of 2 c/s. This fact indicates that there is one proton in the adjacent carbon atom (5-position in formula Ih) of the hemiacetal proton (6-position in formula Ih) and that the dihedral angle of these two protons is close to 90°.[21] Further, from the presence of a singlet at 6·80–7·89 τ corresponding to the proton of —C(—)—CH—CO—O— in the compounds possessing a γ-lactone ring (IV, methyl ester of V, XXVI, XXIX, XXXII, XLI, LIV and LVII in Table 1), formed by the oxidation of this hemiacetal ring, it may be assumed that both carbon atoms adjacent to the carbon atom in the 5-position are tertiary.

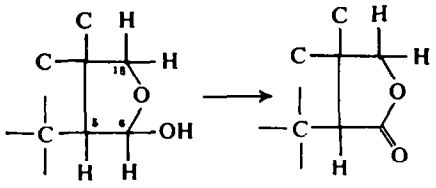
On the other hand, the NMR spectrum of the two protons on the carbon atom at 18-position, bonded to the oxygen atom in the hemiacetal ring, shows the typical AB-type split (VI, VIII, XXVI, XXIX, XLI, XLIV, XLV′, LVII and the methyl ester of LIX in Table 1) and shows two doublets with the coupling constant of ca. 10 c/s at

* The shift of the γ-lactone bands to a lower wave number is probably due to intermolecular hydrogen bonding with hydroxyl group, although the spectra were determined in a nujol mull.

[20] J. A. Pople, W. G. Schneider and H. J. Bernstein, *High Resolution Nuclear Magnetic Resonance* p. 395. McGraw-Hill, New York (1959). Cf. also Ref. 8.

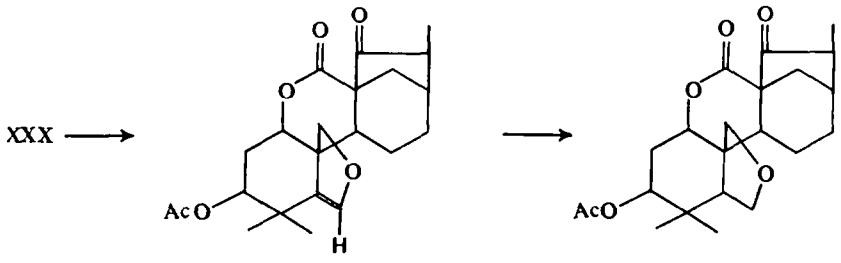
[21] S. Fujiwara, N. Nakagawa and H. Shimizu, *High Resolution Nuclear Magnetic Resonance* p. 78. Maruzen, Tokyo (1961) in Japanese; See also, M. J. Martell, Jr., T. O. Soine and L. B. Kier, *J. Am. Chem. Soc.* **85**, 1022 (1963).

around 5·9 τ. These protons at 18-position appear as a singlet in enmein diacetate (LXVII), methyl ester of V, and in XXX and XXXI, but this is considered to be due to the two protons accidentally having equal values.[22] From these facts, the structure of B_1 ring and its neighborhood may be expanded to the partial structure Ih.



Ih

Chemical evidence supporting the partial structure Ih may be found in the deacetylation reaction of dihydroenmein diacetate (XXX). Heating of XXX under reduced pressure results in the liberation of one mole of acetic acid to form XXXIX, $C_{22}H_{28}O_6$, the IR spectrum of which shows an absorption for a double bond at 1620 cm^{-1} and the NMR spectrum indicates a singlet at 3·58 τ corresponding to the α-proton of a cyclic vinyl ether[23] but no other proton signal for a double bond. Catalytic reduction of XXXIX gives the dihydro compound (XL). The formation of the unsaturated compound (XXXIX) supports the formula Ih in which there is one hydrogen atom in the carbon at 5-position.



XXXIX XL

(4) *Presence of* gem-*dimethyl group and relationship between* A *and* B_1 *rings*

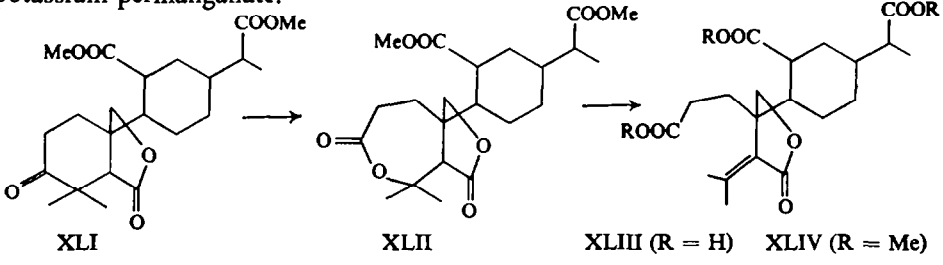
The presence of a *gem*-dimethyl group in enmein is presumed from the formation of II by baryta dry-distillation of enmein[7] and the IR absorptions at 1370 and 1392 cm^{-1} (KBr) in enmein. Comparison of the NMR signals of the two methyl groups on the tertiary carbon atom in the compounds XLVIII and L, to be described later, indicates that the signals are at 9·02 and 9·1 τ in XLVIII and those in L are shifted to the lower magnetic field of 8·85 and 8·96 τ. This fact suggests that there is a *gem*-dimethyl group

[22] For similar examples, cf. D. H. R. Barton, H. J. Chenng, A. D. Cross, L. M. Jackman and M. Martin-Smith, *J. Chem. Soc.* 5061 (1961); R. C. Cookson, A. Melera and A. Morrison, *Tetrahedron* **18**, 1320 (1962).

[23] D. H. R. Barton, H. J. Chenng, A. D. Cross, L. M. Jackman and M. Martin-Smith, *J. Chem. Soc.* 5061 (1961).

on the carbon atom adjacent to the six-membered ring ketone.[24] These assumptions were proved by the following experiments.

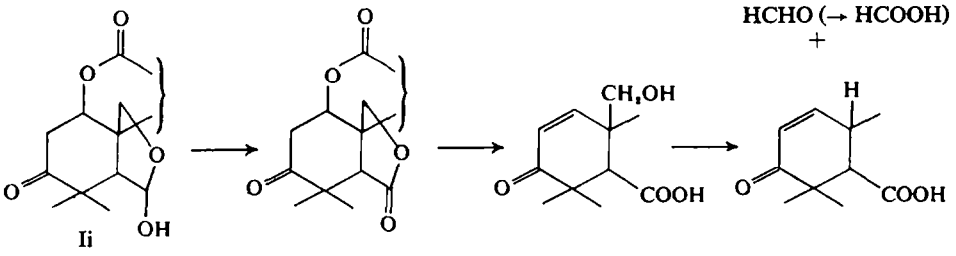
The dihydro ester (XLI), obtained by the catalytic reduction of the dimethyl ester (XXVI), was oxidized with perbenzoic acid in the presence of a catalytic amount of *p*-toluenesulfonic acid and the seven-membered ring lactone (XLII) formed as an intermediate was hydrolyzed with alkali, by which a crystalline tribasic acid (XLIII), $C_{20}H_{28}O_8$, was obtained. The trimethyl ester (XLIV) of XLIII shows IR absorptions at 1743 for a conjugated γ-lactone, and at 1645 cm^{-1} for a double bond and its UV absorption maximum (236 mμ) indicates that the double bond conjugated to this lactone is tetrasubstituted.[25] The NMR spectrum (Table 1) of XLIV indicates signals (7·73 and 8·05 τ) for two methyl groups bonded to a double bond. Consequently, the *gem*-dimethyl group in XLI had changed into an isopropylidene group in XLIII. The shift of the signal of one of these two methyl groups into a lower magnetic field suggests that the double bond and the lactone carbonyl are conjugated in the form of an s-*cis* type.[26] The presence of an isopropylidene group was proved by the formation of acetone by ozonolysis of XLIV. The presence of a *gem*-dimethyl group is also supported by the formation of dimethylmalonic acid by the oxidation of VIII with potassium permanganate.



XLI XLII XLIII (R = H) XLIV (R = Me)

Treatment of bisdehydrodihydroenmein[9] (IV) and its derivatives (V and VIII) with 1N alkali gives formaldehyde and formic acid. This reaction may be understood as the liberation of masked primary alcohol on the carbon atom at 18-position, as shown below, by the retroaldol reaction. In fact, formaldehyde and formic acid are not formed by alkali treatment of dihydroenmein (III) having no carbonyl at 3-position or of IX without a double bond.

From these results, the relationship between the A ring and B_1 ring (hemiacetal ring) was clarified as indicated by the partial structure Ii.



Ii

[24] L. M. Jackman, *Application of NMR Spectroscopy in Organic Chemistry* p. 53. Pergamon Press, London (1959).

[25] A. T. Nielsen, *J. Org. Chem.* **22**, 1539 (1957).

[26] Ref. 21, p. 352.

(5) *Relationship between* B_2 *ring and* C/D *ring*

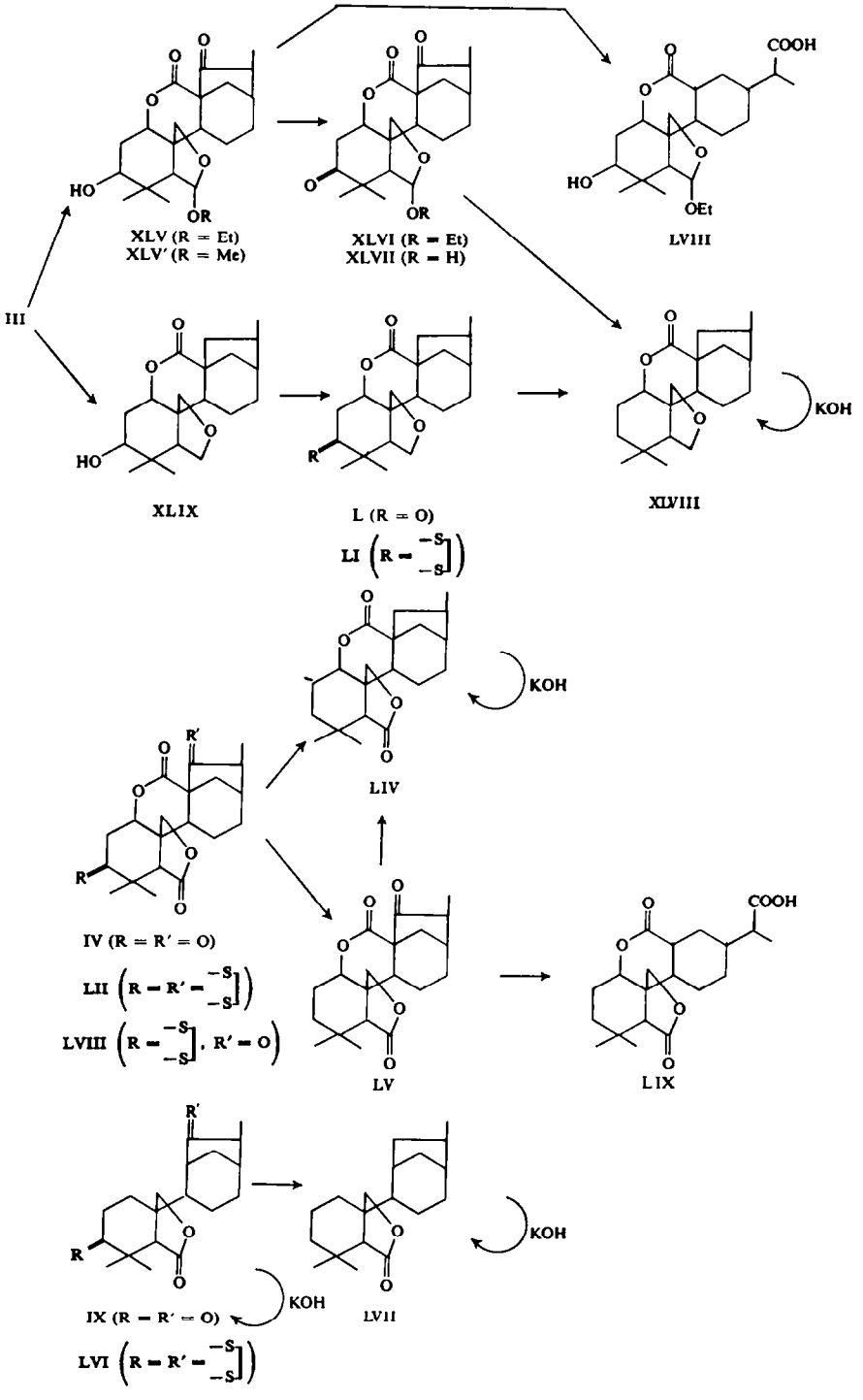
As already stated in Section (2), treatment of bisdehydrodihydroenmein (IV) or the acid (V) with strong acid results in the cleavage of a five-membered ring ketone to form the dicarboxylic acids (XXIV and XXV). This fact indicates that one of the two lactone-carbonyl groups in IV is bonded to the ketone group in D ring at β-position. The fact that the carbonyl group of δ-lactone is bonded to the β-position of the ketone group in D ring is clear from the partial structure (Ii) given above. At the initial stage of the structural determination of enmein, however, it was necessary to determine which lactone group in IV takes part in the β-position of the ketone group in D ring and this was clarified through the following experiment.

Deethylation of O-ethyldihydroenmeinone (XLVI), obtained by the chromic acid oxidation of O-ethyldihydroenmein[8] (XLV), gives dihydroenmeinone (XLVII), which can also be obtained by the chromic acid oxidation of dihydroenmein in pyridine. Conversion of XLVII into its thioketal and desulfuration with Raney nickel results in concurrent reduction of the hemiacetal and the two ketones, and a monolactone (XLVIII) is formed. On the other hand, reduction of the five-membered ring ketone and the hemiacetal in dihydroenmein (III) through its thioketal gives XLIX, which affords the monoketone (L) on oxidation characterized as its oxime and the thioketal (LI). Desulfuration of LI over Raney nickel gives the foregoing monolactone (XLVIII).

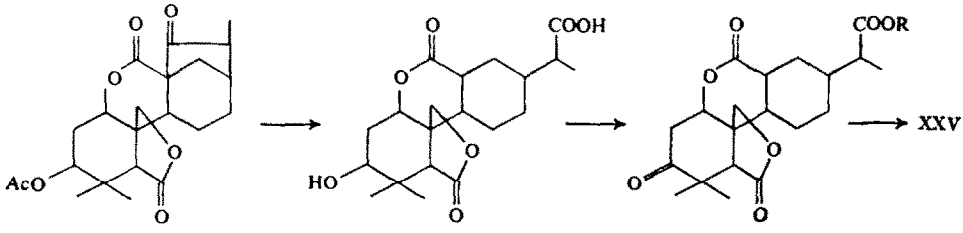
On the other hand, treatment of bisdehydrodihydroenmein (IV) with ethanedithiol gives a dithioketal (LII) as an oily substance and a monothioketal (LIII) as a crystalline substance. Desulfuration of LII over Raney nickel gives a dilactone (LIV), $C_{20}H_{28}O_4$, and its IR spectrum shows absorptions at 1778 (γ-lactone) and 1728 (δ-lactone) cm^{-1}. Desulfuration of LIII gives a monoketodilactone (LV), $C_{20}H_{26}O_5$, the IR spectrum of which shows absorptions at 1780–1745 (γ-lactone and five-membered ring ketone) and 1720 (δ-lactone) cm^{-1}, and the rotatory dispersion curve indicates a negative Cotton effect. A similar transformation of IX to the dithioketal (LVI) and its desulfuration gives a monolactone (LVII), $C_{19}H_{30}O_2$, the IR spectrum of which shows absorption for γ-lactone at 1763 cm^{-1}.

Heating IX, XLV, XLVIII, LIV, LV and LVII with 5% potassium hydroxide results in acid cleavage of the five-membered ring ketone in only XLV and LV, which possess both the five-membered ring ketone and δ-lactone originally present in enmein, and they respectively form the acids LVIII and LIX. Starting materials are recovered unchanged from the other compounds (IX, XLVIII, LIV and LVII). The fact that the five-membered ring ketone with a lactone–carbonyl group in β-position had undergone acid cleavage is clear from the evidence that LIX shows a negative plane curve although its parent compound (LV) shows a negative Cotton effect in its rotatory dispersion curve.

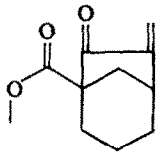
An example of the acid cleavage of a five-membered ring ketone can also be seen in the alkali treatment of XXXII derived from dihydroenmein diacetate (XXX). Hydrolysis of XXXII gives an acid (LX) the oxidation of which with chromic acid forms the acid LXI. Treatment of its methyl ester (LXII) with hydrochloric acid results in the formation of the afore-mentioned dicarboxylic acid (XXV).


III
XLV (R = Et)
XLV' (R = Me)
XLVI (R = Et)
XLVII (R = H)
LVIII
XLIX
L (R = O)
LI
XLVIII
KOH
LIV
IV (R = R' = O)
LII
LVIII (R' = O)
LV
LIX
IX (R = R' = O)
LVI
LVII

These reactions may be represented by the following sequence.



XXXII LX LXI (R = H) LXII (R = Me)

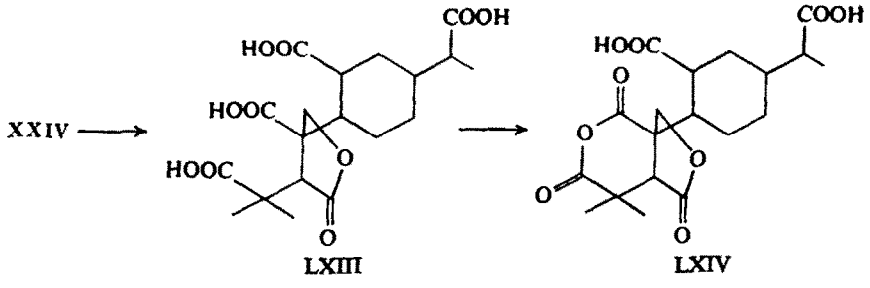


Ij

From these facts, the partial structure of the C/D ring in enmein may be expanded to Ij, and its combination with the partial structure Ii gives the plain structure Ie for enmein.

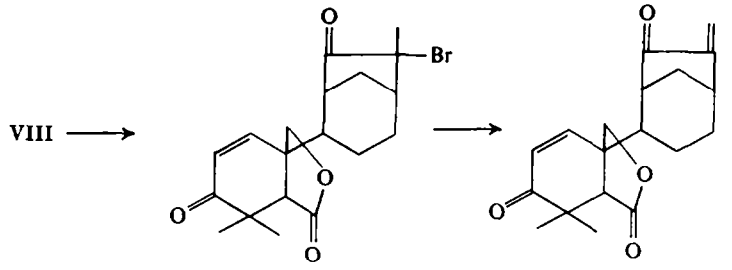
(6) *Other reactions*

Drastic degradations of enmein or its derivatives were tried. Nitric acid oxidation of enmein gives succinic acid, and permanganate oxidation of VIII affords dimethylmalonic acid. On the other hand, mild oxidation of the dicarboxylic acid (XXIV) with potassium permanganate gives a tetracarboxylic acid (LXIII) of 19 carbon atoms, and this acid forms an anhydride (LXIV) on heating.



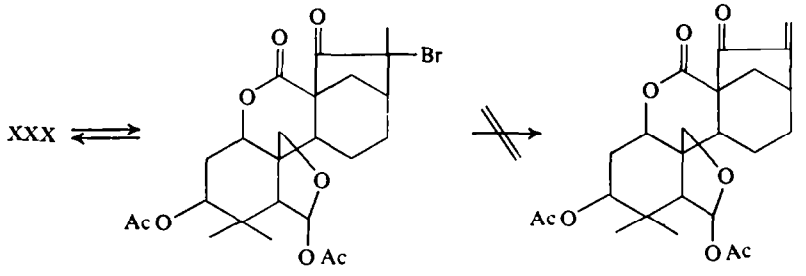
LXIII LXIV

Bromination of VIII with N-bromosuccinimide gives a monobromo compound (LXV) the dehydrobromination of which with lithium chloride affords LXVI. The differential curve of the UV spectra of LXVI (λ_{max} 231 mμ) and VIII (λ_{max} 226 mμ) gives a curve with absorption maximum at 234 mμ which agrees with the absorption maximum (232·5 mμ) of enmein. Direct dehydrogenation of VIII with selenium dioxide also give LXVI which may be assumed to have been formed by dehydrogenation of α,β-positions in the five-membered ring ketone.



LXV LXVI

Enmein obtained from the natural sources invariably contains dihydroenmein (III) and pure enmein has not been isolated to date.[8] In order to obtain enmein diacetate[3] (LXVII) by the application of the above reaction, the following experiments were carried out. Bromination of dihydroenmein diacetate (XXX) with N-bromosuccinimide gives a monobromo compound (LXVIII) the catalytic reduction of which regenerates XXX, and dehydrobromination of LXVIII gives a compound (LXIX), $C_{20}H_{26-28}O_7$. This compound shows UV absorption maximum at 232 mμ the same as enmein but it is different from enmein diacetate and its structure is still not clear.



LXVIII LXVII

(7) *Stereochemistry of enmein*

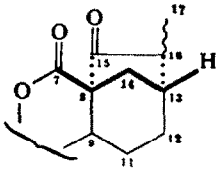
(i) C/D *Ring*. From the structure of the C/D ring, bonding of carbon atoms at 8–15 and 13–16 must take a *cis*-1,3-diaxial configuration with respect to the C ring. Consequently, 8–7 bonding must be equatorial to the C ring. The ORD curve of dihydroenmein (III) shows a negative Cotton effect.[4] If the substituents at 8- and 9-position carbon atoms in the C/D ring have no great influence on this Cotton effect, absolute configuration of the C/D ring in III should be represented as Ik, as in phyllocladene and kaurene.[27,28]

(ii) A/B_1 *Ring*. As has already been mentioned in Section (3), the NMR spectra of various derivatives of enmein show small coupling constant (0–2 c/s) of the hydrogen atoms at 6- and 5-position carbon atoms, and this fact suggests that the dihedral angle of these hydrogen atoms is approximately 90°. In order for these hydrogen atoms to take such a dihedral angle, the A/B_1 rings must be *cis*-bonded and the

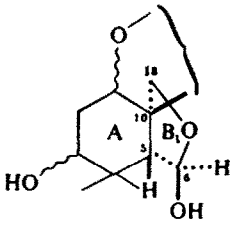
[27] W. Klyne, *Tetrahedron* **13**, 29 (1961).
[28] R. Henderson and R. Hodges, *Tetrahedron* **11**, 226 (1960).

bonding of 10–18 carbon atoms must be axial and that of the 5–6 carbon atoms equatorial with respect to the A ring.

The fact that pyrolysis of dihydroenmein diacetate (XXX) results in facile deacetylation to form a vinyl ether (XXXIX) also satisfies the foregoing relation. If deacetylation by pyrolysis were to occur by *cis*-elimination, XXX must have a steric configuration which allows acetoxyl at 6-position and hydrogen at 5-position to take the *syn*-parallel plane structure. Consequently, steric configuration of the A/B_1 rings would be represented by II or its antipode. In this steric structure, the 10–9 carbon bond would be equatorial and the carbon–hydrogen bond at 5-position have an axial configuration in respect to the A ring.

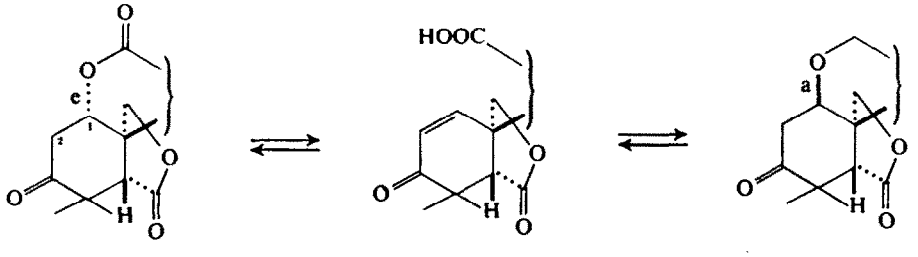


Ik



II

(iii) *Steric structure of carbon atom at 1-position.* Treatment of bis-dehydrodihydroenmein (IV) with hydrogen bromide in acetic acid gives, besides the acid (V) and recovered IV, a neutral substance (LXX), isomeric with IV. From the spectral data of LXX and the formation of V from LXX by treatment with dilute alkali, LXX and IV must be epimeric at the 1-position carbon atom. Treatment of the acid (V) with boron trifluoride gives IV, V and LXX as an equilibrium mixture in 14:21:30 ratio. Treatment of the acid (V) with various inorganic or organic acids also gives IV and LXX in various ratios (Experimental), but its treatment with acetic acid results in formation of LXX alone.



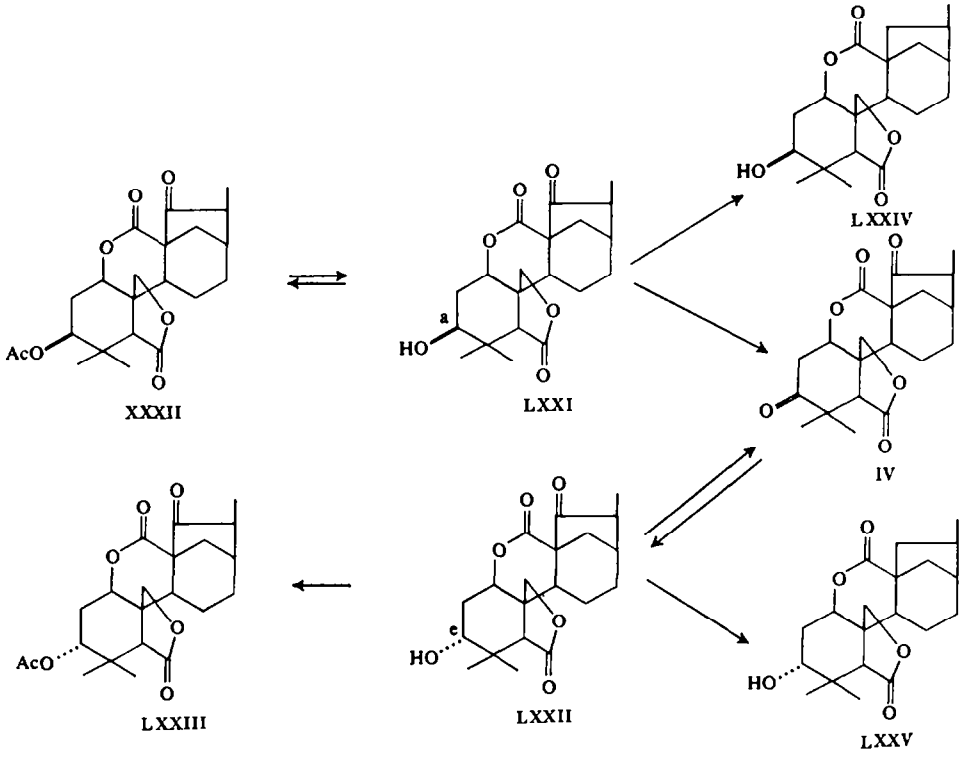
IV V LXX

The NMR spectra (Table 1) of the proton of 1-position carbon atom in IV and LXX show a quartet (J_{AX} = 11·76 c/s, J_{BX} = 6·28 c/s) at 4·75 τ in IV and a quartet at 4·73 τ (J_{AX} = 2·45 c/s, J_{BX} = 3·68 c/s) in LXX. There is not much difference in chemical shift in these two but, while the difference between J_{AX} and J_{BX} in the NMR spectrum of IV is great, that in the spectrum of LXX is small and is close to a triplet.

Consequently, it may be assumed that the C—H bond at 1-position has an axial conformation in IV and equatorial conformation in LXX.[29]

Conversion rate of these two enantiomers into the acid (V) in a dilute alkaline solution as estimated by the application of UV spectroscopy showed that LXX undergoes conversion at about twice the rate of V. This isomerization is thought to occur through *trans*-elimination between masked hydroxyl group at 1-position carbon and hydrogen atom on 2-position carbon, it may be concluded that the C—O bond at 1-position in LXX, with faster isomerization rate, takes the axial conformation and that in IV takes the equatorial conformation in agreement with the result of the above NMR analyses.

(iv) *Hydroxyl group at 3-position carbon atom.* As stated above, oxidation of dihydroenmein 3-monoacetate (XXXI) with chromic acid in acetic acid gives XXXII which can also be obtained by oxidation of dihydroenmein diacetate (XXX) with chromic acid. Hydrolysis of XXXII with dilute hydrochloric acid or dilute alkali results in the formation of dehydrodihydroenmein (LXXI), $C_{20}H_{28}O_6$, in which the ketone group in the C ring remains without undergoing acid cleavage. LXXI is also formed, together with bisdehydrodihydroenmein (IV) and dihydroenmeinone (XLVII), by the oxidation of dihydroenmein (III) with chromic acid in pyridine. LXXI reverts to XXXII by acetylation, and is oxidized to a bisdehydro compound (IV) by oxidation

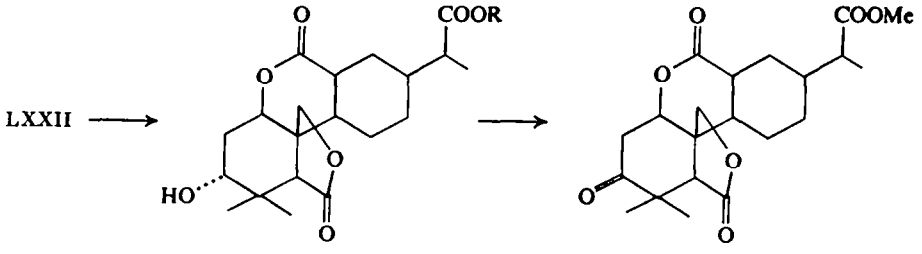


[29] R. U. Lemieux, R. K. Kullnig, H. J. Bernstein and W. G. Schneider, *J. Am. Chem. Soc.* **80**, 6098 (1958); K. L. Williamson and W. S. Johnson, *Ibid*, **83**, 4623 (1961).

with chromic acid in acetic acid. From these results, it is known that the hydroxyl group at 3-position carbon atom in LXXI has the same configuration as that in enmein. On the other hand, reduction of bisdehydrodihydroenmein (IV) with sodium borohydride or catalytic reduction gives only LXXII, $C_{20}H_{26}O_6$, isomeric with LXXI. LXXII forms an acetate (LXXIII) different from XXXII by acetylation, while chromic acid oxidation gives the bisdehydro compound (IV) indicating that LXXI and LXXII are epimeric with respect to the 3-position carbon atom.

In general, reduction of an unhindered ketone with sodium borohydride is preferential under the influence of thermodynamic control and an equatorial alcohol is formed.[30] Based on this fact the hydroxyl in 3-position carbon atom in LXXII is presumed to have an equatorial conformation and that in 3-position carbon atom in LXXI, an axial conformation. This assumption was supported by the result of the NMR spectra of the proton at 3-position carbon atom in the two acetates XXXII and LXXIII. The signal of this proton appears as a diffused triplet at 4·99 τ ($J_{AX} = J_{BX} = 2{\cdot}7$ c/s) in XXXII and as a quartet at 5·43 τ ($J_{AX} = 5{\cdot}6$ c/s, $J_{BX} = 11{\cdot}5$ c/s) in LXXIII indicating that the conformation of the C—H bond is equatorial in XXXII and axial in LXXIII.[29]

Presence or absence of hydrogen bonding between the 3-hydroxyl and the 1-lactone oxygen was examined in LXXIV and LXXV, obtained respectively by reduction of the carbonyl group in the D ring from LXXI and LXXII through thioketal. IR spectra of the two compounds in dil carbon tetrachloride solution showed only absorption for a free hydroxyl (3630 cm^{-1}). Cleavage reaction of the γ-lactone in LXXII was attempted in order to examine the steric relation between the 3-hydroxyl group in the A ring and the γ-lactone formed by the oxidation of the hemiacetal ring in enmein. Treatment of LXXII with sodium methoxide in methanol resulted in recovery of the starting compound when treated in the cold but heat application caused methanolysis of the ketone group in the D ring to form LXXVI which was characterized as its monoacetate and formed a monoketone (LXII) on oxidation with chromic acid. Hydrolysis of LXXVI with alkali under drastic conditions gave a potassium salt which was converted into its silver salt. The IR spectrum of this salt still indicated the presence of a γ-lactone ring and acidification of the salt gave the acid LXXVII. Thus, it may be concluded that the γ-lactone in this molecule is fairly resistant to hydrolysis.

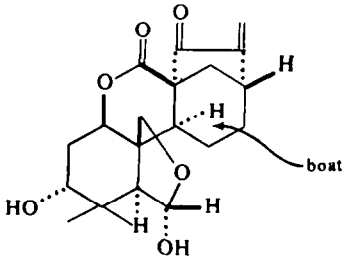


LXXVI (R = Me)
LXXVII (R = H)

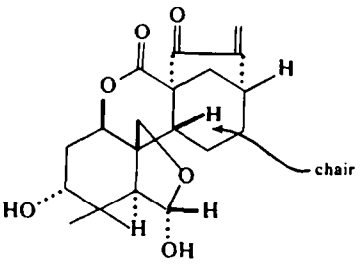
LXII

[30] W. G. Dauben, G. J. Fonken and D. S. Noyce, *J. Am. Chem. Soc.* **78**, 2579 (1956).

(v) 9–10 *Carbon bonding.* Based on the foregoing, the steric structure of enmein was constructed on the Dreiding model and it was found that chair-type and boat-type conformations were possible for the C ring. If the 9–10 carbon bonding were axial in respect to the C ring, this structure would be stereochemically impossible either with the chair or boat form of the C ring, from the nonbonded interaction of the atoms on the A/B_1 ring and on the C/D ring. From such a point of view, there are four possible steric structures for enmein; structures If and Im in which the C ring takes the boat form and 9–10 bonding is equatorial, and the structures Ig and In in which the C ring takes a chair form and 9–10 bonding is equatorial.



Im



In

(vi) *Absolute configuration.* Absolute configuration at the 3-position was determined by the application of Prelog's atrolactic acid method[31] to dehydrodihydroenmein (LXXI). Reaction of LXXI with phenylglyoxalyl chloride gives the phenylglyoxalate (LXXVIII) treatment of which with methyl magnesium iodide and hydrolysis of its product affords a levorotatory atrolactic acid, which had also been obtained by the same method from dihydrogibberellic acid.[32] This has proved the absolute configuration of the 3-hydroxyl in enmein as having a β-configuration, as shown by If and Ig. This absolute configuration agrees well with the result obtained by the application of Klyne's rotation rule[33] to dehydrodihydroenmein (LXXI) and its 3-epimer (LXXII). The difference in molecular optical rotation between LXXI and its acetate (XXXII) is $+64°$ and between LXXII and its acetate (LXXIII) is $-83°$, indicating that they respectively have β- and α-configuration. Steric configuration of the 3-position in enmein would be retained in LXXI and XXXII, so that the hydroxyl group in 3-position of enmein would take a β-configuration.

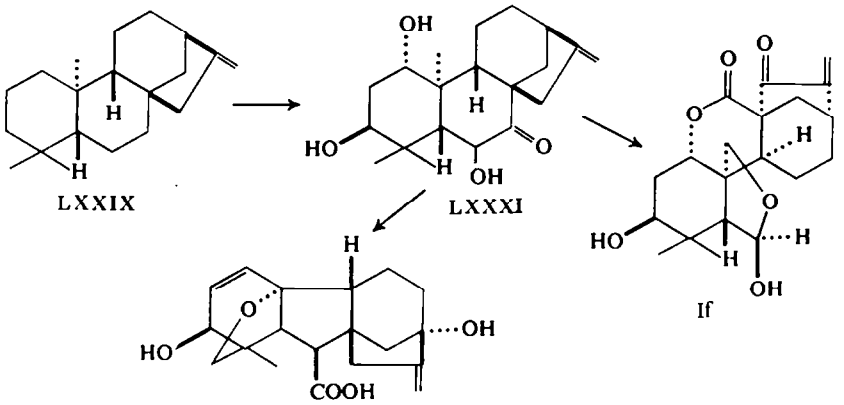
The result of X-ray crystallographic analysis of a dihydroenmein derivative carried out by Iitaka and Natsume[10] indicated that enmein has a structure represented by If or its antipode. Consequently, the absolute configuration of enmein has now been determined as the structure represented by If.

(8) *Biogenesis*

From its steric structure, enmein may be considered as a (−)-kaurene[34] (LXXIX) type, and is one of a few examples (fujenal and fujenoic acid[34a]) of tricarbocyclic

[31] V. Prelog, *Helv. Chim. Acta* **36**, 308 (1953).
[32] S. Masamune, *J. Am. Chem. Soc.* **83**, 1515 (1962).
[33] C. Djerassi, P. A. Hart and E. J. Warawa, *J. Am. Chem. Soc.* **86**, 78 (1964).
[34] M. L. Briggs, B. F. Cain, R. C. Cambie, B. B. Davis, P. S. Ruttlege and J. K. Wilmshurst, *J. Chem. Soc.* 1345 (1963).
[34a] B. E. Cross, R. H. B. Galt and J. R. Hanson, *J. Chem. Soc.* 2937 (1963).

diterpenes formed by the cleavage of a B ring in the kaurene skeleton. Birch *et al.*[35] assumed that gibberellic acid (LXXX) was formed by either the Favorsky rearrangement of its precursor, 6-hydroxy-7-oxokaurene, or by the benzilic acid rearrangement of its 6,7-dioxo compound. In the case of biogenesis of enmein, it is likely that enmein was formed by the oxidative cleavage of the 6–7 carbon bonding in its precursor (LXXXI).



EXPERIMENTAL

Experiments (a) were carried out at Osaka City University and (b) at Kyoto University. M.ps were determined in capillary tubes in (a) and on a hot stage in (b), and they were uncorrected. Microanalyses were done at Microanalytical Laboratories of Faculty of Science, Osaka City University, and of Faculty of Pharmaceutical Science, Kyoto University. NMR spectra were taken with a Varian A60 spectrophotometer and measured in $CHCl_3$ or $CDCl_3$, unless otherwise indicated. Solutions were dried over anhydrous Na_2SO_4.

Decarboxy compound VIII[b]

Compound IV[a] (1 g) was distilled at 245–250° and 1 mm. The distillate crystallized upon cooling, and was recrystallized from EtOH to give colorless rods (650 mg) which were identical with the sample prepared by pyrolysis of V. λ_{max}^{EtOH} 225 mμ (ε 10,000). ν_{max}^{Nujol} 1770 (γ-lactone), 1760 (five-membered ketone), 1724 (δ-lactone), 1678 cm^{-1} (C=C—C=O). (Found: C, 72·44; H, 7·58. Calc. for $C_{19}H_{24}O_4$: C, 72·12; H, 7·65%.)

Methyl ester of V[b]

To a solution of V[a] (100 mg) in MeOH (15 ml) ethereal diazomethane was added. After the mixture was kept 10 min, the excess diazomethane was decomposed by adding a few drops of acetic acid and the solvent evaporated. The residue was taken up in $CHCl_3$ and the solution washed successively with 10% Na_2CO_3aq and water and dried. Evaporation of the solvent gave a crystalline solid (100 mg) which was recrystallized from EtOH to give the methyl ester of V as needles, m.p. 249–250°. ν_{max}^{Nujol} 1770 (γ-lactone), 1740 (five-membered ketone), 1710 (ester), 1680 (conjugated carbonyl). λ_{max}^{EtOH} 224 mμ (ε 9,900). (Found: C, 67·21; H, 7·04. Calc. for $C_{21}H_{26}O_6$: C, 67·36; H, 7·00%.)

Dihydroester VI[b]

The methyl ester (50 mg) of V was hydrogenated in EtOH with 5% Pd–C (0·5 g). After removal of catalyst by filtration, the filtrate gave VI (50 mg) as needles from EtOH, m.p. 218–220°. ν_{max}^{Nujol} 1775–1755, 1714 cm^{-1}. (Found: C, 66·95; H, 7·77. Calc. for $C_{21}H_{28}O_6$: C, 67·00; H, 7·50%.)

[35] A. J. Birch, *The Biosynthesis of Terpenes and Steroids* p. 253. Churchill, London (1959); A. J. Birch, R. W. Richards, H. Smith, A. Harris and W. B. Whalley, *Tetrahedron* 7, 241 (1959).

Dihydro derivative IX *of* VIII[b]

The decarboxy compound VIII (320 mg) was hydrogenated in EtOH (60 ml) at room temp with 5% Pd–C (1 g). One equiv. volume H_2 was absorbed very rapidly in 5 min. After removal of the catalyst by filtration, the filtrate was concentrated to dryness to give IX (320 mg) which crystallized from EtOH as needles, m.p. 80°. ν_{max}^{Nujol} 1769–1740, 1728 cm^{-1}. (Found: C, 71·87; H, 8·31. Calc. for $C_{19}H_{26}O_4$: C, 71·67; H, 8·23%.)

Alkaline hydrolysis of the dihydro derivative IX[b]

A suspension of IX (400 mg) in 5% KOHaq (30 ml) was heated on a water bath for 4 hr. This suspension gradually changed to a clear solution on heating. After cooling, the solution was diluted with water and washed with $CHCl_3$. The aqueous layer was acidified with dil HCl and extracted with ether. The etheral extract was washed with 10% Na_2CO_3aq and water, dried and concentrated to dryness to give IX (365 mg). The alkaline washings were acidified with dil HCl and extracted with ether. Evaporation of ether also afforded the starting material (11 mg).

Pyrolysis of the decarboxy compound VIII[b]

Decarboxy compound VIII (754 mg) was divided into three portions and each was submitted to pyrolysis by heating in a sealed tube under red. press (0·06 mm) at 350–360° for 30 min in a metal bath. After cooling, the product was taken up in ether which was washed successively with 10% Na_2CO_3aq and 10% NaOHaq and separated into neutral, Na_2CO_3-soluble and phenolic fractions. Each fraction was worked up as described below.

(i) *Isolation of 6-methyl-7-oxobicyclo*[3.2.1]*oct-2-ene* XIII and *6-methyl-7-oxobicyclo*[3.2.1]*-octane* (XII). The neutral etheral fraction was washed with water, dried and evaporated to give a volatile oil (382·4 mg) which was chromatographed in ether on alumina. Six 30 ml ethereal fractions were eluted. Fraction 1 gave a volatile oil (236 mg) which showed absorption bands in the IR spectrum at 1730 (five-membered ketone) and 1633 cm^{-1} (double bond).

A 2,4-dinitrophenylhydrazine solution was added dropwise to the oil in EtOH until formation of the precipitate was complete. After standing overnight, the precipitate (352 mg) was collected by filtration and was recrystallized 4 times from EtOH and then collected by filtration and was recrystallized 4 times from EtOH and then 2 times from ethyl acetate to give 2,4-dinitrophenylhydrazone of XII (2·1 mg) as reddish orange scales, m.p. 180–186°. (Found: C, 56·88; H, 5·99; N, 17·33. Calc. for $C_9H_{14}{\cdot}C_6H_4O_4N_4$: C, 56·59; H, 5·70; N, 17·60%.)

The mother liquor was evaporated and the residue chromatographed in $CHCl_3$ on silica gel. Elution with $CHCl_3$ afforded a crystalline solid (185 mg) which was recrystallized 5 times from EtOH to yield 2,4-dinitrophenylhydrazone of XIII (20 mg) as reddish orange scales, m.p. 136–140°. (Found: C, 57·12; H, 5·56; N, 18·12. Calc. for $C_9H_{12}{\cdot}C_6H_4O_4N_4$: C, 56·96; H, 5·10; N, 17·71%.)

Hydrogenation of the oil (496 mg) obtained by concentration of the foregoing fraction 1 in MeOH (40 ml) over 10% Pd–C (1 g) at room temp for 30 min, removal of the catalyst and evaporation of the solvent gave a volatile oil (261·6 mg) which showed IR band at 1730 cm^{-1} (five-membered ketone).

The 2,4-dinitrophenylhydrazone (150·4 mg) of this ketone had m.p. 180–186° (from EtOH) and was identical with that of XII. The semicarbazone was prepared in EtOH and had m.p. 185–189° (from benzene) and $[\alpha]_D^{30}$ +106·57° (*c*, 1·76 in $CHCl_3$). NMR: 8·85 (doublet, J = 6·5 c/s, C_6–CH_3). (Found: C, 61·69; H, 8·78; N, 21·66. Calc. for $C_9H_4{\cdot}CH_3ON_3$: C, 61·51; H, 8·78; N, 21·52%.)

(ii) *Isolation of 7,7-dimethyl-1,6-dioxo-4,5,6,7-tetrahydrophthalan* (XI). Fractions 2-6 of the chromatogram of the neutral portion was rechromatographed over a column of silica gel which was eluted with $CHCl_3$. Evaporation of the solvent afforded a gummy material (32 mg) which was crystallized by trituration with ether. Recrystallization from ether gave XI as colorless rods, m.p. 95–98°, b.p. 150–158°/25 mm (bath temp). λ_{max}^{EtOH} 215 mμ (ε 9,600). $\nu_{max}^{CHCl_3}$ 1753 (α,β-unsaturated γ-lactone), 1719 (six-membered ketone), 1678 cm^{-1} (double bond). NMR: 8·59 $\left(C_7{<}^{CH_3}_{CH_3}\right)$, 7·26 $\left(C_4{<}^{H}_{H}{-}C_5{<}^{H}_{H}\right)$, 5·24 τ (−$CH_3$—OCO). (Found: C, 66·84; H, 6·77. Calc. for $C_{10}H_{12}O_3$: C, 66·65; H, 6·71%.)

(iii) *Isolation of 6-hydroxy-7-methyl-1-oxophthalan* (X) *and a substance of m.p.* 120–129°. The preceding Na_2CO_3aq extract was acidified with conc HCl and the resulting precipitate was extracted with ether which was washed with water, dried and evaporated to give the mixture of a crystalline and an oily substance. The solution of this residue in a mixture of AcOEt–$CHCl_3$ (1:20) was applied to a

column of silica gel which was eluted with the same solvent. Evaporation of the eluate afforded a crystalline substance (15 mg) which was recrystallized from AcOEt to give X as prisms, m.p. 193–197°.

This compound was insoluble in $NaHCO_3$aq and soluble in $NaCO_3$aq with warming. ν_{max}^{KBr} 3390. (hydroxyl), 1727 (γ-lactone connected with benzene), 1615, 1511 cm^{-1} (double bond of benzene) NMR in pyridine: 7·15 (Ph—CH_3), 4·92 τ (—CH_2—O). (Found: C, 66·24; H, 5·17. Calc. for $C_9H_8O_3$: C, 65·85; H, 4·91%.)

When the column was further eluted with AcOEt–$CHCl_3$ (1:6), a crystal (5 mg), m.p. 120–129°, was obtained. It was not further investigated as a minor component.

(iv) *Isolation of* 2,4-*xylenol.* The preceding NaOHaq extract was acidified with conc HCl and extracted with ether. The etheral solution was washed with water and dried. Evaporation of ether gave an oily residue (93·4 mg) which was purified by column chromatography on silica gel. Elution with $CHCl_3$ and evaporation of the solvent afforded an oily material (29·7 mg). 3,5-Dinitrobenzoyl chloride (200 mg) was added to a solution of this oil in pyridine (2·5 ml). The mixture was allowed to stand overnight at room temp and was poured into water. The aqueous solution was extracted with $CHCl_3$ and the solution washed successively with 10% HClaq, 10% Na_2CO_3aq and water. After the $CHCl_3$ extract was dried, the solvent was evaporated to give a crystalline solid (48·7 mg). It was recrystallized from EtOH to give white needles, m.p. 153–156°, which were identical with 2,4-xylyl-3,5-dinitrobenzoate in mixed m.p. and comparison of their IR spectra. (Found: C, 56·79; H, 4·04. Calc. for $C_8H_9O{\cdot}C_7H_3O_6N_2$: C, 56·96; H, 3·82%.)

Ethyl 3-*Ethoxycarbonylcyclohyxylideneacetate* (XV)[b]

Ethoxycarbomethyldiethyl phosphite[36] (13·2 g) was added dropwise to a suspension of NaH (50% oil coating; 2.8 g) in abs benzene (100 ml) with stirring in an ice-water bath. After the mixture was stirred for 1 hr, a solution of XIV[13-5] (9·9 g) in benzene was added dropwise over a period of 30 min. After stirring at room temp for 1 hr the reaction mixture was diluted with water, and then extracted with ether. The extract was washed with water and dried. Evaporation of the solvent gave an oil (11·1 g) which, after distillation, afforded a colorless liquid (7·81 g), b.p. 139–141°/7 mm. (Found: C, 65·43; H, 8·49. Calc. for $C_{13}H_{20}O_4$: C, 64·98; H, 8·39%.)

Ethyl 3-*ethoxycarbonylcyclohexylacetate* (XVI)[b]

Compound XV (2·66 g) was hydrogenated over PtO_2 (70 mg) in EtOH at room temp. Uptake of the equiv of H_2 ceased after 4 hr of hydrogenation. The catalyst was removed by filtration and evaporation of the solvent gave an oil (2·6 g) which, after distillation, gave a colorless liquid (2·38 g), b.p. 138–141°/9·5 mm. (Found: C, 65·20; H, 9·29. Calc. for $C_{13}H_{22}O_4$: C, 64·44; H, 9·15%.)

cis-3-*Carboxycyclohexylacetic acid* (XVII)[b]

A solution of XVI (2·89 g) in 10% methanolic KOH (100 ml) was refluxed for 3 hr. The mixture was concentrated *in vacuo*, diluted with water and washed with benzene. The aqueous layer was acidified with conc HCl and the resulting precipitate was extracted with AcOEt which was washed with water and dried. Evaporation of the solvent gave a crystalline solid (1·76 g) which was recrystallized first from benzene and then from ether–light pet. ether (2:8) as white needles, m.p. 153–156° (Lit.[37] m.p. 153–156°). (Found: C, 58·19; H, 7·71. Calc. for $C_9H_{14}O_4$: C, 58·05; H, 7·58%.)

A mixture of *cis*-3-carboxycyclohexaneacetic acid (295 mg) and aniline (2 ml) was refluxed for 3 hr. The precipitate formed by cooling was completed by addition of ether (50 ml) and then collected by filtration (148 mg). Recrystallization from EtOH gave dianilide of XVII as white needles, m.p. 266–267°. (Found: C, 74·98; H, 6·95; N, 8·60. Calc. for $C_{21}H_{26}ON_2$: C, 74·97; H, 7·19; N, 8·33%.)

7-*Oxobicyclo*[3.2.1]*octane* (XVIII)[b]

Calcium hydroxide (700 mg) and XVII (800 mg) was mixed with water (0·5 ml) with slight warming. After drying, the resultant powder was sealed in four tubes at 0·3 mm and heated in a metal bath at 400–450° for 1·5 hr. After cooling, the products were combined by means of ether and 10% HClaq. The ethereal solution was washed successively with 10% Na_2CO_3aq and water and dried. After evaporation of the solvent, volatile oil (500 mg) was obtained. $\nu_{max}^{CHCl_3}$ 1726 cm^{-1} (five-membered ketone). To a solution of this oil (100 mg) in EtOH (3 ml), a solution of semicarbazide hydrochloride

[36] Cf. W. S. Wadsworth, Jr. and W. D. Emmons, *J. Amer. Chem. Soc.* **83**, 1733 (1961).
[37] F. Ramirez and J. W. Sargent, *J. Amer. Chem. Soc.* **74**, 5785 (1952).

(100 mg) and AcONa (110 mg) in water (5 ml) was added. The mixture was kept overnight at room temp and finally refluxed for 3 hr. After evaporation of the solvent *in vacuo*, water was added. The product was extracted with $CHCl_3$ and was washed with water and dried. Evaporation of the solvent afforded an oily product (97 mg) which was dissolved in benzene and the solution was applied to an alumina column. The desired compound was eluted with $CHCl_3$ and evaporation of the eluate gave a crystalline substance (80 mg) which was recrystallized from benzene, yielding semicarbazone of XVIII as colorless needles, m.p. 187–190° (Lit.[16] m.p. 190–192°). (Found: C, 60·15; H, 8·36; N, 23·43. Calc. for $C_9H_{15}ON_3$: C, 59·64; H, 8·34; N, 23·19%.)

6-Formyl-7-oxobicyclo[3.2.1]*octane* (XIX)[b]

Sodium hydride (50% oil coating; 150 mg) and HCOOEt (1 ml) were added to a stirred solution of XVIII (163 mg) in abs benzene (50 ml). The reaction mixture was stirred at room temp for 2 hr and then refluxed for 4 hr. After standing overnight, an additional NaH (50% oil coating; 150 mg) and HCOOEt (1 ml) were added and the mixture was further refluxed for 5 hr. After cooling, the excess NaH was decomposed by addition of water. The reaction mixture was extracted with water and then with 10% NaOHaq. The combined aqueous solution was washed with benzene and acidified with conc HCl. The resulting precipitate was extracted with ether which was washed with water and dried. Evaporation of the ether gave a reddish brown oil (104 mg). λ_{max}^{EtOH} 270 mμ. $\nu_{max}^{CHCl_3}$ 1736 (five-membered ketone), 1710 (formyl), 1680 (hydrogen bonded, conjugated five-membered ketone), 1603 cm^{-1} (conjugated double bond).

It was converted into its monosemicarbazone in the manner as described in the preparation of XVIII. Recrystallization from benzene gave white scales, m.p. 187–188°. ν_{max}^{KBr} 1678 (NH_2CONH—), 1647 (double bond), 1576 cm^{-1} (—C=N). NMR: 5·31 (1H, OH), 3·28 τ (1H, C=C(H)—OH). (Found: C, 57·57; H, 7·41; N, 20·41. Calc. for $C_9H_{13}O{\cdot}CH_3ON_3$: C, 57·40; H, 7·23; N, 20·08%.)

6-Formyl-6-methyl-7-oxobicyclo[3.2.1]*octane*(XX)[b]

Compound XIX (200 mg) was dissolved in acetone (20 ml) and then MeI (2 ml) and K_2CO_3 (400 mg) was added. The mixture was heated under reflux for 14 hr. After cooling, the remaining K_2CO_3 was removed by filtration, and the filtrate was evaporated to dryness. The residue was dissolved in ether which was washed successively with 10% NaOHaq and water. After evaporation of the solvent an oily substance (196 mg) was obtained. $\nu_{max}^{CHCl_3}$ 2850, 2725 (CH of formyl), 1731 (five-membered ketone), 1715 (formyl), 1634 cm^{-1} (enol ether).

Since examination of the IR spectrum of this oily substance indicated that it contained XX and XXIa, the following treatment was performed to remove the contamination of XXIa.

The oily substance (196 mg) in EtOH (10 ml) and 10% HClaq (8 ml) was heated under reflux for 1 hr. The solvent was evaporated and the residue was extracted with ether. The ethereal solution was washed with 10% NaOHaq and then with water and dried. The solvent was evaporated to give an oily substance (102 mg). $\nu_{max}^{CHCl_3}$ 2850, 2725 (CH of formyl), 1731 (five-membered ketone), 1715 cm^{-1} (formyl). Crystallization of its semicarbazone was not successful.

The reaction of 6-formyl-6-methyl-7-oxobicyclo[3.2.1]*octane* (XX) *with alkali*[b]

(i) A solution of XX (1·6 g) in 1% methanolic KOH (45 ml) was refluxed for 25 min. The solvent was evaporated *in vacuo* and the residue was extracted with ether. The ethereal extract was washed with water and dried. The solvent was evaporated to give an oily material (1·54 g) which was found to be identical with the starting material by comparison of their IR spectra.

The alkaline aqueous washing was acidified with conc HCl and the resulting material was extracted with ether. Evaporation of the solvent gave an acidic oil (48 mg) which exhibited an identical IR spectrum with that of XXII described below.

(ii) A solution of XX (63 mg) in 1% methanolic KOH (14 ml) was heated under reflux for a longer time than the above condition (50 min). The mixture was worked up as described above. The starting material (14 mg) was recovered unchanged and the acidic portion gave an oily substance (43 mg).

The reaction of 6-formyl-6-methyl-7-oxobicyclo[3.2.1]*octane* (XX) *with acid*[b]

Compound XX (72 mg) was dissolved in glacial AcOH (5 ml) and then 10% HClaq (6 ml) was added. The mixture was heated in a water bath for 4·5 hr, diluted with water and extracted with ether.

The organic layer was washed successively with 10% Na_2CO_3aq and water and dried. The solvent was evaporated to give an oil (7·1 mg). The IR spectrum of this material was identical with that of the starting material.

The alkaline washings were acidified with conc HCl and the resulting compound was extracted with ether. The extract was washed with water and dried. The solvent was evaporated to give an oily substance (53·4 mg) which exhibited an identical IR spectrum with that of XXII described below.

Methyl-(3-carboxycyclohexyl)-acetaldehyde (XXII)[b]

A solution of XX (318 mg) in 10% methanolic KOH (20 ml) was refluxed for 1 hr. The mixture was concentrated *in vacuo*. Water was added to the residue, which was washed with ether. The ethereal solution was extracted with 10% Na_2CO_3aq. All alkaline solutions were combined. After acidification, the resulting material was extracted with ether. Evaporation of the solvent gave an oily substance (288 mg). $\nu_{max}^{CHCl_3}$ 1705 cm^{-1} (—COOH, CHO).

Methyl-(3-carboxycyclohexyl)-acetic acid (XXIII)[b]

Nickel peroxide (3 g) was added to a solution of XXII (288 mg) in 2·5% NaOHaq. After stirring at room temp for 6 hr, nickel peroxide was filtered off. The filtrate was washed with ether, acidified with conc HCl and the resulting product was extracted with ether. The ethereal solution was washed with water and dried. The solvent was evaporated to give an oily substance (208 mg) which was chromatographed in $CHCl_3$ on silica gel. $\nu_{max}^{CHCl_3}$ 1706 (COOH) cm^{-1}. Attempt to crystallize the dianilide of this oily material was unsuccessful.

6-Methyl-7-oxobicyclo [3.2.1] *octane* (XII)[b]

(i) Calcium hydroxide (400 mg) and XXIII (400 mg) were mixed with water (0·5 ml) with slight warming on a water bath. After drying, the resultant powder was sealed in a tube at 0·3 mm and heated in a metal bath at 400–450° for 1·5 hr. After cooling, ether and 10% HClaq was added to the contents of the tube. The product was extracted with ether and the ethereal solution was washed successively with 10% Na_2CO_3aq and water. The dried ethereal solution was evaporated to give an oily substance (140 mg). $\nu_{max}^{CHCl_3}$ 1730 cm^{-1} (five-membered ketone).

It was converted into its semicarbazone (10 mg) in the manner as described in the preparation of XVIII. Recrystallization from benzene gave colorless rods, m.p. 181–186°, identical with semicarbazone of XII obtained from the natural product by comparison of their IR spectra in $CHCl_3$. (Found: C, 61·75; H, 8·96; N, 21·36. Calc. for $C_9H_{14}{\cdot}CH_3ON_3$: C, 61·51; H, 8·78; N, 21·52%.)

(ii) To a solution of XIX (281 mg) in pyridine (5 ml) was added *p*-toluenesulfonyl chloride (440 mg). After 30 min, a solution of benzylmercaptan (258 mg) in pyridine (3 ml) was added and the mixture was kept overnight at room temp and then poured into water. The aqueous solution was extracted with ether and the extract was washed successively with 10% NaOHaq and water and dried. After evaporation of the solvent, an oily XXIb (252 mg) was obtained. It was chromatographed in chloroform on silica gel but attempts to crystallize this oily product were unsuccessful. $\nu_{max}^{CHCl_3}$ 1702 (conjugated five-membered ketone), 1593 cm^{-1} (double bond of benzene).

Raney Ni (4 g) was added to a solution of this oily XXIb (230 mg) in EtOH (60 ml) and the mixture was refluxed for 10 hr. The Ni was filtered off and to this filtrate was added a solution of semicarbazide hydrochloride (400 mg) and AcONa (400 mg) in water (7 ml), and the mixture was heated under reflux for 10 hr. The mixture was worked up as described in the preparation of XVIII and recrystallization from benzene gave the semicarbazone (130 mg) of XII as colorless rods, m.p. and mixed m.p. 181–186°.

Dicarboxylic acids, XXIV *and* XXV, *from bisdehydrodihydroenmein* (IV)[a]

(i) *With hydriodic acid.* A mixture of IV[a] (5 g), AcOH (30 ml) and HI (b.p. 128°, 75 ml) was refluxed for 3 hr and then evaporated to dryness. The residual solid (3·4 g) was washed with water, dried and crystallized from MeOH to yield XXIV (1·6 g) as prisms, m.p. 298·5–299° (dec). pKa_1 (Methyl Cellosolve–water, 8:2):6·22. pKa_2:7·82. ν_{max}^{Nujol}: ca. 2300, 1772, 1704, 1687, 1655 cm^{-1}. λ_{max}^{EtOH} 228 mμ (ε 8,150). Neutralization equivalent: 196, 195 (Calc. for $C_{20}H_{26}O_7$ (2 COOH): 189). Saponification equivalent: 156 (N/5 NaOH–MeOH, at room temp overnight), 152 (N/60 NaOH–MeOH, refluxing for 2 hr), 129 (N/3 NaOH–MeOH, refluxing for 2 hr) (Calc. for $C_{20}H_{26}O_7$ (2 COOH and 1 lactone): 126). (Found: C, 63·44; H, 7·07. Calc. for $C_{20}H_{26}O_7$: C, 63·48; H, 6·93%.)

The mother liquor residue from the above crystals was fractionally recrystallized from MeOH to yield XXV (400 mg) as crystals, m.p. 268–269°, which were identical with the acid XXV obtained below (ii), by comparison of their IR spectra.

(ii) *With hydrochloric acid.* A mixture of IV (2 g), AcOH (80 ml) and conc HCl (35 ml) was treated as described in (i). Fractional recrystallization of the products yielded XXIV (1·09 g), m.p. 302–303° (dec), and XXV (0·22 g), m.p. 268°. The latter showed the following physical properties. ν_{max}^{Nujol}: ca. 2310, 1760, 1695, 1685, 1650 cm^{-1}. λ_{max}^{EtOH} 227 mμ (ε 8,450). (Found: C, 63·65; H, 6·93. Calc. for $C_{20}H_{26}O_7$: C, 63·48; H, 6·93%.)

A similar treatment of a mixture of V (0·50 g), AcOH (30 ml) and HCl (8 ml) gave XXIV (0·21 g), m.p. 299–300° (dec).

Ethyl ester of the acid V *from bisdehydrodihydroenmein* (IV)[a]

A mixture of IV (85 mg), conc H_2SO_4 (1·5 ml) and abs EtOH (20 ml) was refluxed for 3 hr. The mixture was evaporated under red. press and most of the H_2SO_4 was neutralized with $NaHCO_3$. The separated solid was collected, washed with water, dried and recrystallized from MeOH to yield the ethyl ester of V (30 mg) as needles, m.p. 219–221° (dec). ν_{max}^{Nujol}: 1773, 1745, 1700, 1690 cm^{-1}. λ_{max}^{EtOH} 225 mμ (ε 9,160). (Found: C, 67·97; H, 7·31. Calc. for $C_{23}H_{28}O_6$: C, 68·02; H, 7·27%.)

Hydrolysis of the ethyl ester of V[a]

(i) *With alkali.* The ethyl ester (96·72 mg) dissolved in EtOH (33 ml) was refluxed with 0·1N NaOHaq (7 ml). After cooling the hydrolyzate was titrated with 0·1N HClaq. Saponification equiv: 190 (Calc. for $C_{22}H_{28}O_6$ with a β-ketoester group). The neutralized solution was evaporated to a small volume and acidified with dil HCl. The resulting solid was collected, washed with water, dried and recrystallized from MeOH to yield XXIV (43 mg), m.p. 300° (dec), whose IR spectrum was identical with that of XXIV obtained above.

(ii) *With acid.* A mixture of the ethyl ester (150 mg), AcOH (10 ml) and conc HCl (2·5 ml) was heated at 110° for 2·5 hr. The mixture was evaporated to dryness and the residual solid was washed with water and dried. The solid was dissolved in $CHCl_3$ and chromatographed on a column containing silica gel (3 g). Elution with $CHCl_3$ yielded a solid (73 mg), which on recrystallization gave prisms (38 mg), m.p. 225–226° (dec), identified as the starting ester by a comparison of the IR spectra. Further elution with $CHCl_3$ yielded a solid (72 mg). Recrystallization from MeOH gave prisms (28 mg), m.p. 300° (dec), identified as XXIV by a comparison of the IR spectra.

Dimethyl ester XXVI[a]

A solution of XXIV (100 mg) in MeOH (30 ml) was treated with an excess of diazomethane solution in ether. The solvent was removed and the residue was recrystallized from MeOH to yield XXVI (75 mg) as needles, m.p. 150–151°. ν_{max}^{Nujol}: 1778, 1732, 1693 cm^{-1}. (Found: C, 65·11; H, 7·56. Calc. for $C_{22}H_{30}O_7$: C, 65·01; H, 7·44%.)

Semicarbazone of the dicarboxylic acid XXIV[a]

The XXIV (100 mg), semicarbazide hydrochloride (150 mg) and anhydrous AcONa (200 mg) were dissolved in a mixture of EtOH (10 ml) and water (4 ml) and the mixture was allowed to stand at room temp for a week. When the mixture was diluted with water and acidified with dil HCl, prisms were deposited. Recrystallization from MeOH gave needles, m.p. 233–236° (dec). (Found: C, 56·55; H, 7·24; N, 9·35. Calc. for $C_{21}H_{29}N_3O_7 \cdot \frac{1}{2}H_2O$: C, 56·72; H, 6·82; N, 9·45%.)

Dimethyl ester XXVII[a]

The XXV was treated with diazomethane as described in the preparation of XXVI. The ester was recrystallized from MeOH to yield needles, m.p. 82–86°. ν_{max}^{Nujol}: 1775, 1720, 1690 cm^{-1}. (Found: C, 64·95; H, 7·57. Calc. for $C_{23}H_{30}O_7$: C, 65·01; H, 7·57%.)

Dihydro derivative of the Dicarboxylic acid XXIV[a]

To a suspension of XXIV (7·0 g) in water (20 ml) was added 1N NaOHaq (36·4 ml). The resulting solution was hydrogenated in the presence of 10% Pd–C (500 mg) at 14°. After the absorption of H_2 (460 ml; 1·5 hr) the catalyst was removed by filtration and the filtrate was acidified with dil HCl.

Precipitates formed were collected by filtration, washed with water and dried (6·67 g). Recrystallization from AcOEt gave the dihydro derivative as needles, m.p. 238° (dec). ν_{max}^{Nujol}: ca. 2680, 1770, 1710–1700 cm^{-1}. (Found: C, 63·31; H, 7·62. Calc. for $C_{20}H_{28}O_7$: C, 63·14; H, 7·42%.)

Dihydro derivative of the dicarboxylic acid XXV[a]

The acid XXV (757 mg) was hydrogenated as described above. The product was recrystallized from AcOEt to yield the dihydro derivative (660 mg) as needles, m.p. 201–203°. ν_{max}^{Nujol}: ca. 2680, 1764, 1715–1692 cm^{-1}. (Found: C, 63·14; H, 7·45. Calc. for $C_{20}H_{28}O_7$: C, 63·14; H, 7·42%.)

Hydroxy-dicarboxylic acid XXVIII[a]

To a solution of XXIV (300 mg) in EtOH (100 ml) was added under ice-cooling a solution of $NaBH_4$ (250 mg) in EtOH (10 ml). The mixture was allowed to stand at 2° overnight, then heated at 60° for 1 hr. The solvent was removed under red. press and the residue was washed with water and recrystallized from MeOH to yield XXVIII (180 mg) as plates, m.p. 266–267° (dec). pKa_1 (Methyl Cellosolve–water, 8:2): 6·37. pKa_2: 7·86. ν_{max}^{Nujol}: 3430, 3580, 2675, 2575, 1747, 1707, 1695, 1747 cm^{-1}. λ_{max}^{EtOH} 205 mμ (ε 2,960). (Found: 60·56; H, 7·84. Calc. for $C_{20}H_{28}O_7 \cdot H_2O$: C, 60·29; H, 7·59%.)

Acetate of the hydroxy-dicarboxylic acid XXVIII[a]

The XXVIII was treated with acetic anhydride and pyridine by the usual method. Recrystallization of the product from MeOH gave the acetate as needles, m.p. 309–310° (dec). ν_{max}^{Nujol}: ca. 2360, 1780, 1764, 1744, 1702 cm^{-1}. (Found: C, 62·48; H, 7·42. Calc. for $C_{22}H_{30}O_8$: C, 62·54; 7·16%.)

Dimethyl ester XXIX[a]

The acetate obtained from XXVIII was treated with diazomethane by the usual method. Recrystallization of the product from MeOH gave XXIX as needles, m.p. 163°. ν_{max}^{Nujol}: 1785, 1772, 1743–1736, 1662 cm^{-1}. (Found: C, 64·13; H, 7·78. Calc. for $C_{24}H_{34}O_8$: C, 63·98; H, 7·61%.)

Dihydroenmein 3-monoacetate (XXXI)[a]

Compound XXX[2,4,5] (5·0 g) and oxalic acid dihydrate (1·5 g) were dissooved in dioxan (100 ml) and water (300 ml). After refluxing for 2 hr, the mixture was concentrated under red. press and crystals separated were collected and dried (3·85 g; 85%), m.p. 238–240°. Specimens crystallized from EtOH–AcOEt showed the same m.p. ν_{max}^{Nujol}: 1762, 1730 (shoulder), 1720, 3500 cm^{-1}. (Found: C, 65·25; H, 7·52. Calc. for $C_{22}H_{30}O_7$: C, 65·01; H, 7·44%.)

Dehydrodihydroenmein monoacetate (XXXII)

(i)[a] To a solution of XXXI (3·85 g) in AcOH (80 ml) was added a solution of CrO_3 (1·5 g) in a mixture of AcOH (20 ml) and a few ml water and the mixture allowed to stand at room temp for 6 hr, then overnight after the addition of excess MeOH. Most of the solvent was removed under red. press and the residue diluted with water. Insoluble solids were collected by filtration, washed with water, dried and recrystallized from dioxan–MeOH to give XXXII (3·33 g) as needles, m.p. 280–285°. From the mother liquor, somewhat impure crystals, were obtained. ν_{max}^{Nujol}: 1780 (shoulder), 1760, 1730, 1720 (shoulder) cm^{-1}. (Found: C, 65·49; H, 7·14. Calc. for $C_{22}H_{28}O_7$: C, 65·33; H, 6·98%.)

(ii)[b] A solution of CrO_3 (110 mg) in AcOH (5 ml) and water (0·5 ml) was added to a solution of XXXI, (220 mg) in AcOH (15 ml). The mixture was allowed to stand overnight at room temp. In order to destroy the excess CrO_3, MeOH (10 ml) was added to the mixture, which was then concentrated to turbidity under red. press at 40°, diluted with water and extracted with $CHCl_3$. The organic layer was washed successively with 10% Na_2CO_3aq and water, and then dried. After evaporation of the solvent, a crystalline material (202 mg) was obtained which was recrystallized from acetone to give colorless prisms of XXXII, m.p. 280–285°. $[\alpha]_D^{22}$ −27·53°; $[M]_D^{22}$ −111·22° (*c*, 0·92 in pyridine). ν_{max}^{Nujol} 1773 (γ-lactone), 1755 (five-membered ketone), 1729 (acetate), 1720 (δ-lactone), 1257 cm^{-1} (acetate). (Found: C, 65·60; H, 7·04. Calc. for $C_{22}H_{28}O_7$: C, 65·34; H, 6·93%.)

(iii)[b] A solution of CrO_3 (40 mg) in AcOH (3 ml) and water (0·1 ml) was added to a solution of XXX (40 mg) in AcOH (5 ml). After being kept for 15 hr at room temp, MeOH was added to the mixture, which was then concentrated to turbidity under red. press, diluted with water and extracted

with $CHCl_3$. The solution was washed successively with 10% Na_2CO_3aq and water, dried and evaporated to dryness.

A crystalline compound (33 mg) obtained was crystallized from acetone to give prisms, m.p. 280–285°, which were identical with XXXII obtained above.

Tetrahydroenmein diacetate (XXXIV)[a]

A solution of XXXIII[3,4,8] (5·46 g) and oxalic acid dihydrate (1·5 g) in dioxan (90 ml) and water (150 ml) was refluxed for 2 hr. Most of the solvent was removed under red. press and the residue was diluted with water. Insoluble solids were collected by filtration, washed with water, dried (4·63 g) and used for the next reaction without further purification.

Dehydrotetrahydroenmein diacetate (XXXV)[a]

The crude XXXIV (0·6 g) obtained above was dissolved in AcOH (5 ml) and a solution of CrO_3 (0·20 g) in AcOH (2 ml) containing a few drops of water was added. After standing at room temp for 5 hr, then overnight with an excess MeOH, XXXV deposited as plates (0·28 g), m.p. 249–250°. From the mother liquor, somewhat impure crystals (0·30 g) were obtained. ν_{max}^{Nujol}: 1781, 1750, 1728 cm^{-1}. (Found: C, 64·45; H, 7·17. Calc. for $C_{34}H_{32}O_8$: C, 64·27; H, 7·19%.)

Sodium borohydride reduction of bisdehydrodihydroenmein (IV) (*diol* XXXVI)[a]

Sodium borohydride (300 mg) was added to a solution of IV (1·4 g) in EtOH under ice-cooling. After standing for 20 min, the mixture was warmed at 55° for 4 hr and then allowed to stand overnight. After the solvent was evaporated under red. press, the residual solid was washed with water, dried (1·0 g), dissolved in $CHCl_3$ and chromatographed through a column containing silica gel (10 g). Elution with $CHCl_3$–MeOH (97:3) yielded crystals, which were recrystallized from MeOH to give XXXVI (580 mg) as crystals, m.p. 258–261°. After drying under red. press at 110° for 8 hr, the m.p. was raised to 263–264°. ν_{max}^{Nujol} 3560, 1766 (γ-lactone), 1710 cm^{-1} (δ-lactone). (Found: C, 66·09; H, 7·79. Calc. for $C_{30}H_{38}O_6$: C, 65·91; H, 7·74%.)

Diacetate of the diol XXXVI[a]

The XXXVI (150 mg) was treated with acetic anhydride and pyridine. The product was recrystallized from benzene–MeOH–water gave the diacetate as plates (150 mg), m.p. 127–130°. (Found: C, 66·79; H, 7·48. Calc. for $C_{34}H_{42}O_8 \cdot \frac{1}{2}C_6H_6$: C, 66·51; H, 7·24%.)

After drying under red. press at 120° for 29 hr, the m.p. was raised to 135–138°. ν_{max}^{Nujol} 1780 (γ-lactone), 1745–1732 cm^{-1} (δ-lactone and acetate). (Found: C, 64·20; H, 7·32. Calc. for $C_{34}H_{38}O_8$: C, 64·27; H, 7·19%.)

Chromium trioxide oxidation of the diol XXXVI[a]

The XXXVI (150 mg) was dissolved in AcOH (5 ml) and mixed with a solution of CrO_3 (150 mg) in AcOH (5 ml) under ice-cooling, and the mixture was allowed to stand at room temp for 4 hr. After the addition of MeOH (4 ml), the mixture was allowed to stand in a refrigerator overnight. Crystals (103 mg) deposited were collected by filtration. Crystals (25 mg) were obtained from the filtrate by evaporation followed by washing of the residue with water. Recrystallization from MeOH gave IV as prisms, m.p. 221–223°. The IR spectrum was identical with that of an authentic sample of bisdehydrodihydroenmein.[8]

Dihydroxy-acid XXXVII[a]

To a solution of V (400 mg) in EtOH (30 ml) was added $NaBH_4$ (300 mg) under ice-cooling. After standing in a refrigerator overnight, the mixture was heated at 60° for 1 hr. The solvent was evaporated under red. press and the residue was washed with water, dried and recrystallized from MeOH–benzene to give XXXVII (240 mg) as needles, m.p. 203–205° (dec). p*Ka* (Methyl Cellosolve–water, 8:2): 6·99. ν_{max}^{Nujol} ca. 3550 (hydroxyl), ca. 3200 (carboxylic acid), 1745 (γ-lactone), 1690 (carboxylic acid), 1650 cm^{-1} (double bond). λ_{max}^{MeOH} 205·5 mμ (ϵ 3,590). (Found: C, 65·88; H, 7·79. Calc. for $C_{30}H_{38}O_6$: C, 65·91; H, 7·74%.)

Diol XXXVIII[a]

The compound VIII (300 mg) was reduced with $NaBH_4$ as described in the preparation of XXXVII. Recrystallization of the product from EtOH gave XXXVIII as crystals, m.p. 201–205°. ν_{max}^{Nujol} ca.

3500 (hydroxyl), 1720 cm^{-1} (γ-lactone). λ_{max}^{EtOH} 205 mμ (ε 2,550). (Found: C, 71·52; H, 9·06. Calc. for $C_{19}H_{28}O_4$: C, 71·22; H, 8·81%.)

Diacetate of the diol XXXVIII[a]

The XXXVIII was acetylated with acetic anhydride and pyridine. Recrystallization of the product from MeOH gave the diacetate as prisms, m.p. 139–142°. ν_{max}^{Nujol} 1755 (γ-lactone), 1735 (shoulder), 1722 cm^{-1} (acetate). (Found: C, 68·26; H, 8·15. Calc. for $C_{23}H_{32}O_6$: C, 68·29; H, 7·97%.)

Pyrolysis of dihydroenmein diacetate (XXX)[b]

Compound XXX (60 mg) was heated at 230–240° for 1 hr under red. press (20 mm). After pyrolysis, distillation of the product at 267–275° (bath-temp) under high vacuum (0·03 mm) gave XXXIX which crystallized from EtOH as prisms, m.p. 175–178°. λ_{max}^{EtOH} 217 mμ (ε 3,700). ν_{max}^{Nujol} 1755, 1732, 1719, 1620 cm^{-1}. (Found: C, 67·79; H, 7·43. Calc. for $C_{22}H_{28}O_6$: C, 68·02; H, 7·27 %.)

Hydrogenation of XXXIX[b]

The XXXIX (10 mg) in EtOH (10 ml) was hydrogenated with Adams' catalyst (15 mg) for 3 hr. The filtered EtOH solution gave XL (10 mg) as needles from EtOH, m.p. 210–212°. ν_{max}^{Nujol} 1706, 1738 cm^{-1}. (Found: C, 67·53; H, 7·82. Calc. for $C_{22}H_{30}O_6$: C, 67·67; H, 7·74%.)

Dimethyl ester XLI[a]

(i) *From the dimethyl ester* XXVI. A solution of XXVI (300 mg) in EtOH (40 ml) was hydrogenated in the presence of 10% Pd-C (300 mg) at 25°, absorbing 20·2 ml H_2 within 10 min. After removal of the catalyst by filtration, the filtrate was evaporated under red. press. The residue was chromatographed through a column containing silica gel (4 g). Elution with $CHCl_3$ yielded a solid, which was recrystallized from MeOH to give XLI (161 mg) as crystals, m.p. 110–113°. ν_{max}^{Nujol} 1765 (γ-lactone), 1735 (ester), 1715 cm^{-1} (six-membered ketone). (Found: C, 64·93; H, 7·81. Calc. for $C_{22}H_{32}O_7$: C, 64·68; H, 7·90%.)

(ii) *From the dihydro derivative of* XXIV. A solution of the dihydro derivative (1·25 g) in MeOH was treated with an ethereal diazomethane solution. The product was recrystallized from MeOH to give XLI as crystals, m.p. 109–110°, which were identical with XLI obtained in (i).

Perbenzoic acid oxidation of the dimethyl ester XLI (*tricarboxylic acid* XLIII)[a]

A mixture of XLI (1·0 g), *p*-toluenesulfonic acid (100 mg) and $CHCl_3$ (30 ml) was mixed with a solution of perbenzoic acid in $CHCl_3$ (7·5 ml; equiv to 615 mg of peracid), and the mixture was allowed to stand at 30° in the dark for 7 days with occasional shaking. The resulting homogeneous mixture was washed with dil KIaq, dil $Na_2S_2O_3$aq and water, successively. The organic layer was shaken with 5% Na_2CO_3aq, then with water, dried and evaporated to give a neutral material. The Na_2CO_3 solution was acidified with dil HCl and extracted with $CHCl_3$. Evaporation of the $CHCl_3$ layer yielded a viscous material as the acidic product. (In the absence of *p*-toluenesulfonic acid the starting ester XLI was recovered (84%).)

The acidic product was dissolved in 0·15N NaOHaq (100 ml) and, after being heated at 80° for 2·5 hr, the mixture was acidified with dil HCl and extracted with AcOEt. The organic layer was washed with water, dried and evaporated. The residual crystals (1·16 g) were suspended in $CHCl_3$ (50 ml) and refluxed for 15 min. Insoluble crystals (430 mg; m.p. 280–283° (dec)) were collected by filtration and recrystallized from AcOEt to give XLIII as prisms (300 mg), m.p. 291° (dec). ν_{max}^{Nujol} 3230–2680 (carboxylic acid), 1725 (unsaturated γ-lactone), 1705 (carboxylic acid), 1645 cm^{-1} (conjugated double bond). λ_{max}^{EtOH} 236 mμ (ε 8,020). (Found: C, 60·48; H, 7·31. Calc. for $C_{20}H_{28}O_8$: C, 60·59; H, 7·12%.)

The neutral material obtained above was dissolved in $CHCl_3$ and chromatographed through a column containing silica gel (5 g). Elution with $CHCl_3$ yielded a viscous material (333 mg), which was dissolved in a mixture of N/6 NaOHaq (60 ml) and EtOH (10 ml). After being heated at 80° for 2·5 hr, the mixture was worked up to give XLIII (150 mg) as prisms, m.p. 288–291°.

Trimethyl ester XLIV *of the tricarboxylic acid* XLIII[a]

The XLIII (300 mg) was dissolved in MeOH and treated with an etheral diazomethane solution. Recrystallization of the product gave XLIV (210 mg) as crystals, m.p. 149–151°. ν_{max}^{Nujol} 1743 (unsaturated γ-lactone), 1735 (methyl ester), 1645 cm^{-1} (conjugated double bond). λ_{max}^{EtOH} 236 mμ (ε 9,480). (Found: C, 62·96; H, 7·98. Calc. for $C_{23}H_{34}O_8$: C, 62·99; H, 7·82%.)

Ozonolysis of the trimethyl ester XLIV[a]

Ozonized O_3 was bubbled through a solution of XLIV (360 mg) in $CHCl_3$ (25 ml) under ice-cooling for 5 hr. The solvent was evaporated under red. press at 20° and the residue was heated at 100° with water (10 ml) in a stream of N_2. The exhausted gas was passed through a 0·3% 2,4-dinitrophenylhydrazine solution in dil HClaq. After 2 hr, an orange precipitate (62 mg) was obtained. Recrystallization from EtOH gave orange crystals (44 mg), m.p. 125–128°, which showed no m.p. depression on admixture with an authentic sample of acetone 2,4-dinitrophenylhydrazone. In a controlled experiment with chloroform, no 2,4-dinitrophenylhydrazone was formed.

The reaction mixture was divided into the neutral fraction (25 mg) and the acidic fraction (120 mg). Attempts to crystallize both fractions were unsuccessful.

Alkali treatment of bisdehydrodihydroenmein (IV)[a,*]

(i) A suspension of IV (500 mg) in 5% NaOHaq was subjected to steam-distillation and the distillate (100 ml) was collected in a solution of dimedon (200 mg) in EtOH (50 ml). Crystals deposited were collected by filtration (16 mg) and recrystallized from aqueous EtOH to give needles (13 mg), m.p. 191–194°, which showed no m.p. depression on admixture with an authentic sample of the formaldehydedimedon adduct.

(ii) A solution of IV (500 mg) in 1N NaOH solution in EtOH (146 ml) was heated under reflux for 3 hr. The solvent was evaporated under red. press. and the residue was acidified with dil H_2SO_4 (1:2) (50 ml) and subjected to steam-distillation. The distillate (250 ml) was neutralized with 0·1N NaOHaq (2·9 ml; equiv to 0·29 mmole formic acid). Paperchromatography (1-butanol–EtOH–3N ammonia, 4:1:5) of the neutralized solution showed a single spot, the R_f-value of which was same as that of formic acid.

Alkali treatment of the acid V[a]

A solution of V (500 mg) in 1N NaOHaq was subjected to steam-distillation. The distillate was treated as described above to yield formaldehyde–dimedon adduct (7 mg), m.p. 191–194°. The residue was acidified with 50% H_2SO_4aq and subjected to steam-distillation. The distillate consumed 0·51 ml of 0·1N NaOHaq (equiv to 0·05 mmole formic acid) and, on paper chromatogram, showed a single spot, the R_f-value of which was same as that of formic acid.

Alkali treatment of the decarboxy derivative VIII[a]

(i) A suspension of VIII (500 mg) in 5% NaOHaq was subjected to steam-distillation, and the distillate (100 ml) was collected in a solution of dimedon in EtOH. From the mixture, formaldehydedimedom adduct (20 mg), m.p. 191–194° was obtained.

(ii) A mixture of VIII (500 mg), NaOH (5·8 g), MeOH (100 ml) and water (46 ml) was heated under reflux for 3 hr. The mixture was concentrated under red. press. and acidified with dil H_2SO_4 to yield a yellow solid, which was collected by filtration and recrystallized from aqueous MeOH as prisms (25 mg), m.p. 288° (dec). ν_{max}^{Nujol} 3600, 1720 cm^{-1}. (Found (after drying *in vacuo* at 60° for 1 hr): C, 66·59; H, 8·15. Calc. for $C_{18}H_{26}O_5$: C, 67·06; H, 8·13%.)

The filtrate obtained above was subjected to steam-distillation. The distillate (200 ml) consumed 0·8 ml (equiv to 0·08 mmole formic acid) of 0·1N NaOHaq and, a paper chromatogram showed a single spot, the R_f-value of which was same as that of formic acid.

O-*Ethyldihydroenmeinone* (XLVI)[b]

Compound XLV[8] (470 mg) was oxidized with Sarett's reagent prepared from CrO_3 (0·5 g) and pyridine (5 ml) at room temp for 24 hr. The mixture was diluted with cold water and extracted with $CHCl_3$. The extract was thoroughly washed with 10% Na_2CO_3aq and water, dried and evaporated under red. press. The product (400 mg) was crystallized from EtOH to yield XLVI as prisms, m.p. 169–170°. ν_{max}^{Nujol} 1763, 1720 cm^{-1}. (Found: C, 67·40; H, 7·85. Calc. for $C_{22}H_{30}O_6$: C, 67·67; H, 7·74%.)

Acetate of O-*ethyldihydroenmein* (XLV)[b]

Compound XLV (0·5 g) was acetylated by the acetic anhydride–pyridine method (24 hr at room temp). The reaction mixture was poured into cold water and extracted with ether. The ethereal

* The authors are indebted to Drs. M. Tomoeda and S. Kanatomo for their suggestion on these experimental conditions.

extract yielded an oily residue (520 mg) which was chromatographed in $CHCl_3$ on silica gel. Several fractions eluted by $CHCl_3$ were found to be the acetate (250 mg) of XLV which crystallized from ether–n-hexane, m.p. 100–101°. ν_{max}^{Nujol} 1762, 1731 cm^{-1}. (Found: C, 66·20; H, 8·14. Calc. for $C_{24}H_{34}O_7$: C, 66·34; H, 7·89%.) Further elution with $CHCl_3$ gave XLV (starting material; 170 mg).

Dihydroenmeinone (XLVII)[b]

A solution of XLVI (200 mg) in AcOH (8 ml) and water (3 ml) was heated on a water bath for 3 hr, then diluted with water and extracted with $CHCl_3$. The extract was washed with 10% Na_2CO_3aq and water, and dried. Evaporation of $CHCl_3$ gave XLVII (112 mg) which crystallized from EtOH as plates, m.p. 241–242°. $[\alpha]_D^{25}$ −43·5° (*c*, 0·64 in $CHCl_3$). ν_{max}^{Nujol} 3499, 1755, 1712 cm^{-1}. (Found: C, 66·10; H, 7·16. Calc. for $C_{20}H_{26}O_6$: C, 66·28; H, 7·23%.)

Oxidation of dihydroenmein (III) *with chromium trioxide-pyridine*[b]

A mixture of III (0·3 g), CrO_3 (0·3 g) and pyridine (3 ml) was allowed to stand overnight at room temp. After working up, a neutral fraction gave a crystalline mass (254 mg) which was chromatographed over silica gel in $CHCl_3$. The first eluate with $CHCl_3$ gave IV (131 mg), m.p. 221–223°. The following eluate with the same solvent gave XLVII (61 mg) as plates from EtOH, m.p. 241–242°. $[\alpha]_D^{25}$ −43·5° (*c*, 0·64 in $CHCl_3$). ν_{max}^{KBr} 3480, 1754, 1711 cm^{-1}. (Found: C, 66·10; H, 7·02. Calc. for $C_{20}H_{26}O_6$: C, 66·23; H, 7·23%.) The mother liquor from the recrystallization of XLVII gave two different kinds of crystals on standing which were separated mechanically. One was XLVII and the other was collected and recrystallized from EtOH to give LXXI (7 mg), m.p. 258–261°. ν_{max}^{Nujol} 3510, 1780, 1761, 1714 cm^{-1}. (Found: C, 65·86; H, 7·37. Calc. for $C_{20}H_{26}O_6$: C, 66·28; H, 7·23%.)

Monolactone XLVIII[b]

A mixture of XLVII (402 mg), ethanedithiol (5 ml) and BF_3-etherate (1 ml) was allowed to stand for 2 days at room temp and poured into 10% Na_2CO_3aq and extracted with $CHCl_3$. The extract was washed with water and dried. Removal of $CHCl_3$ gave an oily residue (620 mg) which was chromatographed over silica gel in $CHCl_3$. Elution with $CHCl_3$ afforded dihydroenmeinone thioketal (530 mg) as an oil. $\nu_{max}^{CHCl_3}$ 1721 cm^{-1}. The oily thioketal was heated with Raney Ni (from the alloy (40 g)) in EtOH under reflux for 10 hr. After removing of Raney Ni by filtration, the filtrate was concentrated to dryness under red. press. to give an oil (96 mg). The oil was chromatographed over silica gel in $CHCl_3$. The first eluate with $CHCl_3$ gave an oil (12 mg) which was not investigated further. The second eluate with the same solvent gave XLVIII (63 mg) which crystallized from ether–n-hexane as prisms, m.p. 179–180°. ν_{max}^{Nujol} 1728 cm^{-1}. (Found: C, 75·61; H, 9·69. Calc. for $C_{20}H_{30}O_3$: C, 75·43; H, 9·50%.)

Deoxodihydroneoenmein (XLIX)[b]

A mixture of III (0·5 g), ethanedithiol (5 ml) and BF_3-etherate (1·5 ml) was allowed to stand for 72 hr at room temp and worked up as described above. A neutral fraction gave an oily residue (780 mg) which was chromatographed over silica gel in $CHCl_3$. The chloroform eluate afforded dihydroenmein thioketal (620 mg) as an oil. The oily thioketal was heated with Raney Ni (prepared from 20 g of the alloy) in EtOH under reflux for 10 hr. After removal of Raney Ni by filtration, the filtrate was concentrated to dryness, under red. press., to give an oily residue. The residue was chromatographed over silica gel in $CHCl_3$. The first $CHCl_3$ eluate yielded an oil (85 mg). The second eluate with the same solvent gave XLIX (52 mg) as needles (from EtOH), m.p. 215–216°. ν_{max}^{Nujol} 3450, 1721 cm^{-1}. (Found: C, 71·73; H, 8·93. Calc. for $C_{20}H_{30}O_4$: C, 71·82; H, 9·04%.) The oily residue obtained from the first eluate was rechromatographed over silica gel in $CHCl_3$ to yield a crystalline compound (4 mg), m.p. 249–250° (from EtOH). (Found: C, 68·91; H, 8·70. Calc. for $C_{10}H_{30}O_5$: C, 68·54; H, 8·63%) and additional amount of XLIV (38 mg).

Oxidation of XLIX *to* L[b]

Compound XLIX (112 mg) was oxidized with CrO_3–pyridine complex for 24 hr. A residue from a neutral fraction was chromatographed over silica gel in $CHCl_3$. Elution with $CHCl_3$ yielded L (63 mg) as plates from EtOH, m.p. 181–184°. ν_{max}^{Nujol} 1730, 1706 cm^{-1}. $\nu_{max}^{CHCl_3}$ 1731, 1720 cm^{-1}. (Found: C, 72·52; H, 8·58. Calc. for $C_{20}H_{28}O_4$: C, 72·26; H, 8·49%.) The oxime of L formed needles, m.p.

260–261°, from EtOH. ν_{max}^{Nujol} 3430, 1728, 1630 cm^{-1}. (Found: C, 69·04; H, 8·54; N, 4·00. Calc. for $C_{30}H_{39}O_4N$: C, 69·13; H, 8·41; N, 4·03%.)

Reduction of L *to the monolactone* XLVIII[b]

The compound L (62 mg) was allowed to stand overnight in ethanedithiol (1 ml) and BF_3-etherate (0·5 ml) at room temp. The reaction mixture was worked up as described above. The neutral fraction (100 mg) was chromatographed over silica gel in $CHCl_3$. The $CHCl_3$ eluate gave LI (71 mg) as needles from ether–EtOH, m.p. 146–149°. ν_{max}^{KBr} 1772 vm^{-1}. (Found: C, 64·20; H, 8·20. Calc. for $C_{32}H_{32}O_3S_2$: C, 64·37; H, 8·35%.) A suspension of LI (60 mg) and Raney Ni (from 8 g of the alloy) in abs EtOH (70 ml) was refluxed for 10 hr and filtered. The filtrate was concentrated to dryness to give XLVIII (17 mg) as needles from EtOH, m.p. 179–180°, which were identical with the sample derived from XLVII as mentioned above.

Treatment of bisdehydrodihydroenmein (IV) *with ethanedithiol in the presence of boron trifluoride*[b]

To a suspension of IV (310 mg) in ethanedithiol (2 ml) was added BF_3-etherate (1 ml). After standing at room temp for 2 days, the reaction mixture was worked up as described before. A residue from a neutral fraction was chromatographed in $CHCl_3$ over silica gel. The first $CHCl_3$ eluate gave LII (300 mg) as an oil. $\nu_{max}^{CHCl_3}$ 1770, 1727 cm^{-1}. The following eluate with the same solvent yielded LIII (80 mg) as colorless needles from acetone–EtOH, m.p. 242–245°. ν_{max}^{KBr} 1780, 1755, 1720 cm^{-1}. $\nu_{max}^{CHCl_3}$ 1760, 1720 cm^{-1}. (Found: C, 60·35; H, 6·51. Calc. for $C_{32}H_{38}O_5S_2$: C, 60·54; H, 6·47%.)

Dilactone LIV[b]

(i) Compound LII (300 mg) was reduced with Raney Ni (from 20 g of the alloy) in EtOH by heating under reflux for 8 hr. After filtration of the mixture, the filtrate gave LIV (63 mg) as prisms from acetone–EtOH, m.p. 235–237°. $[\alpha]_D^{25}$ −89·9° (*c*, 1·03 in $CHCl_3$). ν_{max}^{Nujol} 1778, 1728 cm^{-1}. (Found: C, 72·49; H, 8·71. Calc. for $C_{30}H_{38}O_4$: C, 72·26; H, 8·49%.)

(ii) The LV (215 mg) was treated with ethanedithiol (1 ml) and BF_3-etherate (0·2 ml) at room temp overnight. The reaction mixture was worked up as mentioned above. Chromatography of the crude product in $CHCl_3$ over silica gel gave the thioketal (360 mg) as a crystalline mass. ν_{max}^{KBr} 1765, 1722 cm^{-1}. This thioketal was heated with Raney Ni (from 12 g of the alloy) in EtOH under reflux for 7 hr. After removal of catalyst by filtration, the filtrate afforded LIV, m.p. 235–237°, which was superimposable in IR spectrum with the sample from (i).

Alkali treatment of LIV[b]

A solution of LIV (50 mg) in 10% KOHaq (10 ml) and EtOH (5 ml) was refluxed for 10 hr. The solution was then diluted with water (10 ml) and washed with $CHCl_3$, acidified with dil HCl and extracted with $CHCl_3$. The extract was washed with 10% Na_2CO_3aq and water and dried. Evaporation of $CHCl_3$ gave LIV (20 mg). The washings with 10% Na_2CO_3aq were acidified again and extracted with $CHCl_3$. The organic layer also gave LIV (22 mg).

Monoketo dilactone LV[b]

Compound LIII (1·2 g) mentioned above was heated in EtOH with Raney Ni (from 50 g of the alloy) under reflux for 10 hr. Filtration and removal of EtOH gave LV (272 mg) as plates from acetone–EtOH, m.p. 300–302°. $[\alpha]_D^{25}$ −132·3°. (*c*, 1·00 in $CHCl_3$), ν_{max}^{KBr} 1780–1745, 1720 cm^{-1}. (Found: C, 69·09; H, 7·5. Calc. for $C_{30}H_{36}O_5$: C, 69·34; H, 7·57%.)

Alkaline hydrolysis of O-*ethyldihydroenmein* (XLV)[b]

Compound XLV (420 mg) was heated in 5% KOHaq (25 ml) on a water bath for 4 hr. The reaction mixture was diluted with water (25 ml) and washed with $CHCl_3$. The aqueous layer was acidified with dil HCl under cooling and extracted with ether. The extract was washed with water and dried. Evaporation of the solvent gave the acid LVIII (380 mg) which crystallized from n-hexane–ether as plates, m.p. 118–120° (dec). ν_{max}^{KBr} 1720–1700 cm^{-1}. (Found: C, 61·56; H, 8·54. Calc. for $C_{32}H_{34}O_7 \cdot H_2O$: C, 61·66; H, 8·47%.)

Alkaline hydrolysis of LV[b]

A suspension of LV (47 mg) in 3% KOHaq was heated on a water bath for 10 hr. The crystals of LV gradually dissolved. After heating, the solution was diluted with water (6 ml) and washed

with $CHCl_3$. The aqueous layer was acidified with dil HCl and extracted with $CHCl_3$. The extract was worked up in usual manner. A neutral fraction gave LV (6 mg). An acidic fraction yielded LIX (40 mg) as colorless needles from EtOH, m.p. 266–267°. ν_{max}^{KBr} 3400 (hydroxyl), 1750, 1732, 1710 cm^{-1}. (Found: C, 66·07; H, 7·96. Calc. for $C_{20}H_{28}O_6$: C, 65·91; H, 7·74%.)

Methyl ester of LIX[b]

The methyl ester prepared with diazomethane in the usual manner formed needles which crystallized from EtOH, m.p. 239–241°. ν_{max}^{KBr} 1785, 1750, 1725 cm^{-1}. (Found: C, 66·84; H, 7·98. Calc. for $C_{21}H_{30}O_6$: C, 66·64; H, 7·99%.)

Monolactone LVII[b]

A mixture of IX (1·05 g), ethanedithiol (4 g) and BF_3-etherate (0·8 ml) was allowed to stand at room temp for 48 hr and worked up to yield an oily residue (1·25 g). This was crystallized from EtOH–$CHCl_3$ to give LVI as prisms, m.p. 193–195°. ν_{max}^{KBr} 1758 cm^{-1}. (Found: C, 58·45; H, 7·25. Calc. for $C_{22}H_{34}S_4O_5$: C, 58·71; H, 7·2%.)

The thioketal (0·9 g) was heated in EtOH (600 ml) with Raney Ni (prepared from 80 g of the alloy) under reflux for 10 hr. After cooling, Raney Ni was removed by filtration. Evaporation of the filtrate under the red. press. yielded an oil which crystallized from n-hexane–ether. Recrystallization with the same solvent gave LVII as needles, m.p. 125–127°. ν_{max}^{KBr} 1759 cm^{-1}, $\nu_{max}^{CHCl_3}$ 1763 cm^{-1}. (Found: C, 78·30; H, 10·59. Calc. for $C_{19}H_{30}O_2$: C, 78·57; H, 10·41%.)

Alkaline hydrolysis of LVII[b]

A solution of LVII (10·4 mg) in 10% KOHaq (8 ml) and EtOH (1 ml) was refluxed for 20 hr. The solution was washed with $CHCl_3$, acidified with dil HCl and extracted with ether. The extract was washed with water and dried. Evaporation of ether gave a crystalline substance (3·2 mg) which was recrystallized from n-hexane to afford the starting material as needles, m.p. and mixed m.p. 125–127°. The washings with $CHCl_3$ were washed with water, dried and evaporated to give the starting material (6 mg), m.p. and mixed m.p. 125–127°.

Hydroxy-acid LX *from dehydrodihydroenmein monoacetate* (XXXII)[a]

A mixture of XXXII (1·62 g), 1N NaOHaq (20·00 ml) and EtOH (80 ml) was heated under reflux for 40 min. The hydrolyzate was neutralized with 1N H_2SO_4aq (saponification equiv: 133–134), then acidified with dil H_2SO_4 and concentrated to yield crystals (1·26 g). Recrystallization from aqueous MeOH gave needles, m.p. 266–267°. ν_{max}^{Nujol} 3600 (shoulder), 3520, 2620–2400, 1783, 1728, 1704, 1635 cm^{-1}. Neutralized equiv: 400 (Calc. for $C_{20}H_{28}O_7 \cdot H_2O$: 398). pK_a (Methyl Cellosolve–water, 8:2): 6·92. (Found: C, 60·55; H, 7·52. Calc. for $C_{20}H_{28}O_7 \cdot H_2O$: C, 60·29; H, 7·59%.)

Methyl ester of the hydroxy-acid LX[a]

This was prepared in the usual manner and recrystallized from MeOH–AcOEt to give needles m.p. 257–260°. ν_{max}^{Nujol} 3630, 1793, 1750, 1735 cm^{-1}. (Found: C, 63·95; H, 7·63. Calc. for $C_{21}H_{30}O_7$: C, 63·94; H, 7·66%.)

Acetate of the hydroxy-acid LX[a]

This was prepared with acetic anhydride and pyridine overnight and recrystallized from MeOH–AcOEt as needles m.p. 246–248°. ν_{max}^{Nujol} 3230 (monomeric carboxylic acid), 1760 (shoulder; γ-lactone), 1750 (shoulder), 1740 (δ-lactone and acetate), 1715 cm^{-1} (carboxylic acid). (Found: C, 62·44; H, 7·17. Calc. for $C_{22}H_{30}O_8$: C, 62·54; H, 7·16%.)

Methyl ester acetate of the hydroxy-acid LX[a]

(i) *From the methyl ester of* LX. Prepared with acetic anhydride and pyridine overnight, the product crystallized from aqueous MeOH as needles m.p. 181–182°. ν_{max}^{Nujol} 1755, 1730 cm^{-1} (shoulder). (Found: C, 63·36; H, 7·36. Calc. for $C_{23}H_{32}O_8$: C, 63·28; H, 7·39%.)

(ii) *From the acetate of* LX. A solution of the acetate (30 mg) in acetone was treated with an ethereal diazomethane solution. After removal of the solvent, the residue was recrystallized from

aqueous MeOH to give needles (16 mg), m.p. 179–181°. The IR spectrum was identical with that of the needles obtained in (i).

Keto-acid LXI[a]

A solution of LX (1·0 g) in AcOH (6 ml) was treated with CrO_3 (0·30 g) in AcOH (2 ml) containing a few drops of water at room temp for 6·5 hr. After the addition of MeOH (10 ml), the mixture was allowed to stand overnight. Evaporation of the solvent followed by dilution with water yielded a solid (0·84 g), which was recrystallized from aqueous MeOH to give needles, m.p. 237–238°. pK_a (Methyl Cellosolve–water, 8:2): 6·82. ν_{max}^{Nujol} 3260 (monomeric carboxylic acid), 1770 (shoulder; γ-lactone), 1740 (δ-lactone), 1700 cm^{-1} (shoulder; carboxylic acid). (Found: C, 63·03; H, 7·19. Calc. for $C_{20}H_{26}O_7$: C, 63·48; H, 6·93%.)

Methyl ester LXII *of the keto-acid* LXI[a]

(i) *From the methyl ester of* LX. To a solution of the methyl ester (0·17 g) in AcOH (2 ml) was added CrO_3 (60 mg) in AcOH (1 ml), and the mixture was allowed to stand for 5 hr. After the addition of MeOH (3 ml), the mixture was allowed to stand overnight, depositing plates (70 mg), m.p. 216–218°. Additional crystals (50 mg) were obtained from the mother liquor. ν_{max}^{Nujol} 1792, 1759, 1732, 1720 cm^{-1} (shoulder). λ_{max}^{EtOH} no maximum above 205 mμ. $\lambda_{max}^{NaOEt-EtOH}$ 228 mμ. (Found: C, 64·47; H, 7·25. Calc. for $C_{21}H_{28}O_7$: C, 64·27; H, 7·19%.)

(ii) *From the hydroxy-acid* LX. A solution of LX (0·30 g) in AcOH (2 ml) was treated with CrO_3 (0·10 g) in AcOH (2 ml) as described. After evaporation of the solvent followed by dilution with water, the mixture deposited a solid (0·25 g), which was dissolved in dioxan–acetone and treated with an ethereal diazomethane solution. The product was chromatographed through a column containing silica gel (4 g). Elution with $CHCl_3$–MeOH (98:2) yielded crystals (0·22 g), which were recrystallized from AcOEt and isooctane to give plates, m.p. 216–218°. The IR spectrum was identical with that of the ester obtained in (i).

Semicarbazone of the methyl ester LXII[a]

A mixture of LXII (0·10 g), semicarbazide hydrochloride (0·20 g), anhydrous AcONa (0·2 g), water (2 ml) and MeOH (15 ml) was allowed to stand overnight. Crystals deposited (52 mg) were collected by filtration and recrystallized from MeOH to give the semicarbazone as needles, m.p. 262° (dec). ν_{max}^{Nujol} 3460, 1745, 1700, 1569 cm^{-1}. λ_{max}^{EtOH} 231 mμ (ε 14,600). (Found: C, 58·86; H, 6·79; N, 9·61. Calc. for $C_{22}H_{31}O_7N_3$: C, 58·78; H, 6·95; N, 9·35%.)

Acid treatment of the methyl ester LXII (*dicarboxylic acid* XXV)[a]

A mixture of LXII (0·36 g) and conc HCl–acetic acid (1:5; 20 ml) was heated under reflux for 3 hr. After removal of the solvent under red. press., the residue was dried over NaOH. Recrystallization from MeOH gave prisms (0·22 g), m.p. 270–271°. The IR spectrum was identical with that of XXV obtained from IV. The m.p. was not depressed on admixture with a sample of XXV.

Oxidation of enmein (I) *with nitric acid*[a]

Powdered enmein (5 g) was added in small portions at 110° to 60% HNO_3 (40 g) containing ammonium vanadate (70 mg). The mixture was heated under reflux until the evolution of nitrogen dioxide ceased (10 hr), then kept at 95–100° for 9 hr. After the mixture was concentrated under red. press., the residue was made alkaline with the addition of $NaHCO_3$ and extracted continuously with ether. The extract was dried and evaporated to yield a viscous liquid (124 mg), which did not crystallize. The bicarbonate solution was acidified with dil HCl and extracted continuously with ether for 46 hr. The extract was dried and evaporated to leave a brown liquid (2·07 g), which on standing for several days deposited crystals (51 mg). The crystals were collected by filtration, washed with ether and recrystallized from AcOEt to give plates (19 mg), m.p. 186–187°, which were identified as succinic acid by a comparison of the IR spectra and by the mixed m.p. determination.

The filtrate from the succinic acid was evaporated and the residue was distilled *in vacuo* to yield a colorless liquid (119 mg), b.p. 50–60°/2 mm, which was identified as acetic acid by VPC. The residue from the distillation was dissolved in EtOH and treated with an ethereal diazomethane solution. After evaporation of the solvent, the residue was distilled *in vacuo* to yield two fractions; (i) b.p. below

130°/2 mm (890 mg) and (ii) b.p. 130–220°/2 mm. The fraction (ii) solidified and was recrystallized from MeOH to give prisms (78 mg), m.p. 209–210°. ν_{max}^{Nujol} 1775, 1750, 1730 cm^{-1}. The UV spectrum showed no maximum above 210 mμ and showed a maximum at 225 mμ after addition of EtONa. Mol. wt. (Rast method): 227, 215. (Found: C, 56·49; H, 5·85. Calc. for $C_{10}H_{12}O_5$: C, 56·60; H, 5·70%; mol. wt., 212.)

The residue was crystallized from MeOH in needles (30 mg), m.p. 229–230°. ν_{max}^{Nujol} 1775 (shoulder) 1770, 1765 (shoulder), 1730, 1725 cm^{-1} (shoulder). Mol. wt. (Rast method): 367. (Found: C, 57·64; H, 6·37. Calc. for $C_{17}H_{22}O_8$: C, 57·62; H, 6·26; mol. wt., 354.)

Oxidation of V *and potassium permanganate to dimethylmalonic acid*[b]

A solution of $KMnO_4$ (10 g) in water (350 ml) was added dropwise under stirring to a cold solution of V (1·44 g) in 0·1N KOHaq (60 ml). After standing overnight at room temp, the mixture was filtered and the MnO_2 washed with hot water. The filtrate, combined with the washings, was acidified with dil HCl and extracted with ether. Chromatography of the acidic products (0·215 g) on silicic acid (6 g) gave in the acetone–$CHCl_3$ (1:10) eluates 18 mg of needles, which were recrystallized from aqueous MeOH. They gave a correct m.p. and satisfactory analysis for dimethylmalonic acid. (Found: C, 45·74; H, 6·34. Calc. for $C_5H_8O_4$: C, 45·45; H, 6·10%.) The *p*-bromophenacyl ester gave no depression upon admixture with synthetic *p*-bromophenacyl dimethylmalonate, m.p. 128–129°.

Oxidation of the dicarboxylic acid XXIV *with potassium permanganate*[a]

Potassium permanganate (1·5 g; equiv to 60 atoms) was added in small portions with stirring to a solution of XXIV (0·90 g) in 5% Na_2CO_3aq (20 ml). After stirring for 3 hr, MeOH (5 ml) was added in order to decompose the unreacted permanganate and the mixture was allowed to stand overnight. The reaction mixture was saturated with SO_2 and extracted continuously with ether for 14 hr. The ether layer was dried and evaporated. The residue was treated with hot $CHCl_3$ (50 ml) and the insoluble solid was collected by filtration. Recrystallization from MeOH gave the LXIII as crystals (165 mg), m.p. 185° (dec) after drying *in vacuo* at 80° for 2 hr. Less pure crystals (145 mg), m.p. 178–180°, were obtained from the mother liquor. ν_{max}^{Nujol} ca. 2650, 1760 (shoulder), 1720–1710, 1625 cm^{-1}. $\nu_{max}^{CHCl_3-NEt_3}$ 1745 (γ-lactone), 1608 cm^{-1} (carboxylate). Neutralization equiv: 118 (Calc. for $C_{19}H_{26}O_{10}\cdot H_2O$ (4COOH): 108). (Found: C, 53·26; H, 6·69. Calc. for $C_{19}H_{26}O_{10}\cdot H_2O$: C, 52·77; H, 6·53%.)

The m.p. was raised to 267–268° (dec) after drying *in vacuo* at 120° for 6 hr. (Found: C, 56·44; H, 6·32. Calc. for $C_{19}H_{26}O_{10}$: C, 56·33; H, 6·15%.)

After further drying *in vacuo* (2 mm) at 140° for 22 hr, the LXIII was converted to LXIV, m.p. 261–264° (dec). ν_{max}^{Nujol} ca. 2670 (carboxylic acid), 1810, 1755 (six-membered anhydride), 1780 (γ-lactone), 1710 cm^{-1} (carboxylic acid).

Tetramethyl ester of LXIII[a]

The LXIII, m.p. 178–180°, (260 mg) was treated with an ethereal diazomethane solution as usual. The product was purified by silica gel chromatography and recrystallized from benzene to give the tetramethyl ester (220 mg), m.p. 137–139°. The m.p. was 140–142° after drying *in vacuo* at 90° for 1 hr. ν_{max}^{Nujol} 1773 (γ-lactone), 1725 cm^{-1} (ester). (Found: C, 59·16; H, 6·88. Calc. for $C_{23}H_{34}O_{10}$: C, 58·71; H, 7·28%.)

Monobromo compound LXV[a]

A mixture of VIII (300 mg), N-bromosuccinimide (170 mg), a few mg of benzoyl peroxide, and $CHCl_3$ (30 ml) was heated under refluxing for 3 hr. The reaction mixture was passed through a column containing silica gel (4 g) twice. Elution with $CHCl_3$ yielded crystals (235 mg), which were recrystallized from MeOH to give LXV as plates (145 mg), m.p. 212–213°. ν_{max}^{Nujol} 1760 (γ-lactone), 1740 (five-membered ketone), 1690 (conjugated ketone), 1625 cm^{-1} (conjugated double bond). (Found: C, 57·72; H, 5·85; Br, 20·36. Calc. for $C_{19}H_{23}BrO_4$: C, 57·70; H, 5·87; Br, 20·23%.)

Compound LXVI[a]

(i) *From the monobromo compound* LXV. A mixture of LXV (570 mg), anhydrous LiCl (182 mg) and dimethylformamide (4 ml) was heated at 110–120° in an atm of N_2 for 4 hr. The mixture was

diluted with water (10 ml) and precipitates formed were collected by filtration, washed with water, dried, dissolved in $CHCl_3$ and chromatographed through a column containing silica gel (7 g). Elution with $CHCl_3$ yielded crystals (343 mg), which was recrystallized from MeOH to give LXVI (320 mg) as crystals, m.p. 182–185°. ν_{max}^{Nujol} 1755 (γ-lactone), 1715 (conjugated five-membered ketone), 1680 (conjugated six-membered ketone), 1640, 1610 cm^{-1} (conjugated double bond). λ_{max}^{EtOH} 231 mμ (ε 15,000). The difference curve between the UV spectra of LXVI and VIII showed a maximum at 234 mμ (ε 8,100). (Found: C, 72·46; H, 7·36. Calc. for $C_{19}H_{22}O_4$: C, 72·59; H, 7·05%.)

(ii) *From the decarboxy compound* VIII. A solution of VIII (300 mg) and SeO_2 (200 mg) in AcOH (15 ml) was heated under reflux. After the solvent was evaporated under red. press., the residue was washed with water, dried, dissolved in $CHCl_3$ and chromatographed through a column containing silica gel. Elution with $CHCl_3$ yielded a solid, which on several recrystallizations from MeOH gave crystals (45 mg), m.p. 170–173°. The IR spectrum was identical with that of LXVI obtained in (i).

Monobromo derivative LXVIII *of dihydroenmein diacetate* (XXX)[a]

A mixture of XXX (550 mg), N-bromosuccinimide (230 mg), a few mg of benzoylperoxide and $CHCl_3$ (30 ml) was heated under reflux for 3 hr. The reaction mixture was passed through a column containing silica gel (7 g) twice. Elution with $CHCl_3$–MeOH (100:1) yielded crystals (490 mg), which were recrystallized from EtOH to give LXVIII (300 mg) as prisms, m.p. 236° (dec). ν_{max}^{Nujol} 1750, 1725 cm^{-1}. (Found: C, 54·80; H, 5·92. Calc. for $C_{24}H_{31}BrO_8$: C, 54·65; H, 5·92%.)

Catalytic hydrogenation of the monobromo derivative LXVIII[a]

A solution of LXVIII (100 mg) and anhydrous AcONa (80 mg) in EtOH (40 ml) was shaken in the presence of 10% Pd–C (100 mg) in an atmosphere of H_2. After the absorption of H_2 (8·1 ml at 16°), the catalyst was filtered off and the filtrate was evaporated. The residue was washed with water, dried and recrystallized from MeOH to give plates (45 mg), m.p. 242–244° (dec), which were identified as XXX by a comparison of the IR spectra.

Dehydrobromination of the monobromo derivative LXVIII[a]

A mixture of LXVIII (500 mg), anhydrous LiCl (120 mg) and dimethylformamide (3 ml) was heated at 110° for 3·5 hr. Precipitates which deposited by the addition of ice-water were collected by filtration, washed with water, dried, dissolved in $CHCl_3$ and chromatographed through a column containing silica gel (5 g). Elution with $CHCl_3$–MeOH (99:1) yielded crystals, which were recrystallized from MeOH to give LXIX as prisms, m.p. 282–283° (dec). ν_{max}^{Nujol} 1750 (conjugated five-membered ketone), 1710 (δ-lactone and acetate), 1640 cm^{-1} (conjugated double bond). λ_{max}^{EtOH} 232 mμ (ε 8,600). (Found: C, 65·54; H, 6·87. Calc. for $C_{22}H_{26}O_7$: C, 65·66; H, 6·51. Calc. for $C_{22}H_{28}O_7$: C, 65·33; H, 6·89%.)

Iodoacetate of dihydroenmein 3-*monoacetate* (XXXI)[a]

A mixture of bromoacetate[10] of XXXI (200 mg), NaI (900 mg) and acetone (50 ml) was heated under reflux for 4 hr. After removal of the solvent, the residue was washed with water, dried, dissolved in $CHCl_3$ and chromatographed through a column containing silica gel (3 g). Elution with $CHCl_3$–MeOH (100:0·5) yielded crystals, which were recrystallized from EtOH to give the iodoacetate (150 mg) as needles, m.p. 224° (dec). ν_{max}^{Nujol} 1750, 1728, 1715 cm^{-1}. (Found: C, 50·40; H, 5·55. Calc. for $C_{24}H_{31}IO_8$: C, 50·18; H, 5·44%.)

Isobisdehydrodihydroenmein (LXX)[a]

A solution of IV (250 mg) in AcOH (70 ml) was saturated with dry HBr under ice-cooling. After standing overnight at room temp, the mixture was evaporated at 55° under red. press. The residue was washed with water, dried and recrystallized from MeOH to give LXX (60 mg) as leaflets, m.p. 205–207°. ν_{max}^{Nujol} 1772 (γ-lactone), 1747 (five-membered ketone), 1725 cm^{-1} (δ-lactone and six-membered ketone). (Found: C, 66·79; H, 6·94. Calc. for $C_{30}H_{34}O_6$: C, 66·65; H, 6·71%.)

From the mother liquor, the starting material (25 mg) and the acid V[8] (45 mg) were obtained by fractional recrystallizations. Both substances were identified by the comparison of the IR spectra.

Treatment of the acid V *with boron trifluoride*[a]

A solution of V[8] (1·12 g) in CH_2Cl_2 (60 ml) was mixed with BF_3-etherate (1 ml). After standing at 30° for 40 hr, the mixture was shaken with water, $NaHCO_3$aq and water, successively, dried and

evaporated under red. press. The residue was fractionally crystallized to give bisdehydrodihydroenmein (156 mg) and the isomer LXX (330 mg). Acidification of the $NaHCO_3$ extract with dil HCl yielded precipitates, which were collected by filtration, washed with water, dried and crystallized from MeOH to give the starting material (234 mg). These products were identified by the comparison of the IR spectra.

Isobisdehydrodihydroenmein (LXX)[b]

(i) Compound V (300 mg) in AcOH (30 ml) was heated under reflux for 10 hr and kept at room temp for 3 days. The solution was evaporated to dryness *in vacuo* and the residue taken up in AcOEt, washed with 10% Na_2CO_3aq and water, dried and evaporated. The resulting crystalline substance (53·3 mg) was recrystallized from EtOH to give LXX as colorless scales, m.p. 205–207°. ν_{max}^{KBr} 1779 (γ-lactone), 1747 (five-membered ketone), 1723 cm^{-1} (δ-lactone, six-membered ketone). (Found: C, 66·37; H, 6·46. Calc. for $C_{30}H_{34}O_6$: C, 66·65; H, 6·71%.)

The alkaline washings mentioned were acidified with dil HCl and extracted with AcOEt, and the extract washed with water. After removal of AcOEt, the starting material (238 mg) was recovered unchanged.

(ii) A mixture of V (605 mg) and conc HCl (1 ml) in AcOH (20 ml) was kept at room temp for 5 days. After removal of the AcOH *in vacuo*, the residue was dissolved in $CHCl_3$. The solution was washed with 10% Na_2CO_3aq and then water, dried and evaporated to give a solid mass (125 mg) which was crystallized from EtOH to afford LXX (80 mg) as scales, m.p. and mixed m.p. 205–207°. Concentration of the mother liquors, and crystallization of the precipitate from EtOH gave IV (25 mg) as needles, m.p. and mixed m.p. 221–223°. The NMR spectrum of the crude product indicated that the ratio of LXX to IV was approximately 2:1. Acidification of the combined alkaline washings with dil HCl followed by extraction with $CHCl_3$ gave the unchanged starting material (465 mg).

(iii) A mixture of V (132 mg) and 57% HIaq (1 ml) in AcOH (7 ml) was kept at room temp for 2 days. The mixture was concentrated, diluted with water and extracted with $CHCl_3$. The extract was washed successively with 10% Na_2CO_3aq, $Na_2S_2O_3$aq and water, dried and evaporated to give a crystalline material (45 mg) which was recrystallized from EtOH to yield LXX (25 mg) as colorless scales, m.p. and mixed m.p. 205–207°. From the mother liquors, there was obtained IV (8 mg), m.p. and mixed m.p. 221–223°. The NMR spectrum of the crude material showed that it was a mixture of products consisting of 11 parts of IV and 20 parts of LXX.

The combined alkaline washings were acidified with conc HCl and extracted with $CHCl_3$. The solution was washed with water, dried and evaporated to give the unchanged starting material (85 mg).

(iv) A mixture of V (200 mg) and conc H_2SO_4 (1·5 ml) in AcOH (7 ml) was kept at room temp for 2 days. The mixture was concentrated to dryness, diluted with water and extracted with AcOEt. The extract was washed with 10% Na_2CO_3aq and water, dried and evaporated. The residue (45 mg) was crystallized from EtOH to yield LXX (23 mg) as scales, m.p. and mixed m.p. 205–207°. From the mother liquor, IV (10 mg) was obtained as needles. From the NMR spectrum of the crude mixture, it was estimated that it consisted of approximately 8 parts of IV and 11 parts of LXX.

From the alkaline washings the starting material (153 mg) was recovered unchanged.

Alkali treatment of isobisdehydrodihydroenmein (LXX)[a]

A mixture of LXX (28 mg), 0·1N NaOHaq (1·4 ml) and MeOH (12 ml) was heated under reflux. After the addition of 0·1N HClaq, the mixture was evaporated under red. press. Recrystallization of the residual crystals from MeOH gave the V (19 mg) as plates, m.p. 233–234° (dec). The IR spectrum was identical with that of an authentic sample of V[a].

Relative rate of conversion of bisdehydrodihydroenmein (IV) *and of isobisdehydrodihydroenmein* (LXX), *into the acid* V[a]

UV spectra of a solution (ca. 1×10^{-4} mole/l; 3 ml) of each compound in EtOH were taken after the addition of 0·001N NaOHaq (0·2 ml). The rate of the formation of V was determined by measurements of the extinction coefficient at the absorption maximum (226 mμ) of V. Yield of V from IV: 4·1% (after 5 min), 15% (after 30 min). Yield of V from isobisdehydrodihydroenmein: 12% (after 5 min), 35% (after 30 min).

Dehydrodihydroenmein (LXXI)[b]

A solution of Na_2SO_3 (1 g) in water (15 ml) was added to a solution of XXXII (150 mg) in EtOH (20 ml), and acetone (8 ml). The mixture was refluxed for 4 hr, concentrated under red. press., and extracted with $CHCl_3$.

The extract was washed with water, dried, and evaporated to give a crystalline material (95 mg) which crystallized from MeOH to afford LXXI as colorless scales, m.p. 258–261°, identical with the sample obtained by oxidation of III with CrO_3–pyridine. $[\alpha]_D^{23}$ −51·18°; $[M]_D^{23}$ −185·27° (*c*, 1·02 in pyridine). ν_{max}^{Nujol} 3510 (hydroxyl), 1780 (γ-lactone), 1761 (five-membered ketone), 1714 cm^{-1} (δ-lactone). (Found: C, 66·28; H, 7·24. Calc. for $C_{20}H_{26}O_6$: C, 66·28; H, 7·23%.)

Hydrolysis of dehydrodihydroenmein monoacetate (XXXII) (*dehydrodihydroenmein* (LXXI) *and its isomer*)[a]

(i) *With hydrochloric acid.* A solution of XXXII (1·1 g) in EtOH (60 ml) and 15% HClaq (25 ml) was refluxed for 7 hr. After the solvent was removed under red. press., the residual solid was washed with water, dried, dissolved in $CHCl_3$ and chromatographed through a column containing silica gel (10 g). Elution with $CHCl_3$–MeOH (100:0·5) yielded crystals, which were recrystallized from EtOH to give recovered XXXII as prisms (240 mg). The IR spectrum was identical with that of XXXII.

Elution with $CHCl_3$–MeOH (100:1) yielded crystals, which were recrystallized from EtOH to give LXXI as prisms (440 mg), m.p. 258–261°. ν_{max}^{Nujol} 3530 (hydroxyl), 1780 (γ-lactone), 1755 (five-membered ketone), 1708 cm^{-1} (δ-lactone). (Found: C, 66·15; H, 7·12. Calc. for $C_{20}H_{26}O_6$: C, 66·28; H, 7·23%.)

(ii) *With alkali.* A solution of XXXII (500 mg) in EtOH (400 ml) and 0·583N NaOHaq (7 ml) was refluxed for 2·5 hr. After cooling, the mixture was acidified with 1N HClaq (5 ml) and evaporated under red. press. The residual solid was washed with water, dried, dissolved in $CHCl_3$ and chromatographed through a column containing silica gel (7 g). Elution with $CHCl_3$–MeOH yielded a solid (340 mg), which was recrystallized from MeOH to give LXXI as prisms (260 mg). The IR spectrum was identical with that of the LXXI obtained in (i).

Elution with $CHCl_3$–MeOH (100:3) yielded a solid (120 mg) which recrystallized from MeOH to give an isomer of LXXI as needles (60 mg), m.p. 244–247°. ν_{max}^{Nujol} 3500 (hydroxyl), 1775 (γ-lactone), 1755 (five-membered ketone), 1705 cm^{-1} (δ-lactone). (Found: C, 66·09; H, 7·21. Calc. for $C_{20}H_{26}O_6$: C, 66·28; H, 7·23%.)

This substance was acetylated with acetic anhydride–pyridine. The product was recrystallized from MeOH to give an isomer of XXXII as needles, m.p. 309° (dec). ν_{max}^{Nujol} 1765 (γ-lactone), 1750 (five-membered ketone), 1717 cm^{-1} (δ-lactone and acetate). (Found: C, 65·35; H, 7·07. Calc. for $C_{22}H_{28}O_7$: C, 65·33; H, 6·98%.)

Acetylation of dehydrodihydroenmein (LXXI)[a]

A mixture of LXXI (0·1 g), acetic anhydride (0·3 ml) and pyridine (0·6 ml) was allowed to stand at room temp overnight. Needles (90 mg) deposited were collected by filtration and washed with EtOH, m.p. 280–285°. The IR spectrum was identical with that of XXXII, m.p. 280–285°, obtained above.

Chromium trioxide oxidation of dehydrodihydroenmein (LXXI)[a]

A solution of LXXI (0·20 g) and CrO_3 (75 mg) in AcOH (3 ml) containing a few drops of water was allowed to stand for 5 hr. After the mixture was kept overnight with an excess of MeOH, the crystals deposited were (0·16 g) recrystallized from MeOH–AcOEt to give prisms, m.p. 221–223°. The IR spectrum was identical with that of IV.[a]

Oxidation of dehydrodihydroenmein (LXXI)[b]

Compound LXXI (50 mg) and CrO_3 (30 mg) in AcOH (12 ml) were kept at room temp overnight. After addition of MeOH, the mixture was concentrated under red. press., diluted with water and extracted with $CHCl_3$. The extract was washed with 10% Na_2CO_3aq and with water, dried and evaporated to give a crystalline material (42 mg), which recrystallized from EtOH to give IV as colorless needles, m.p. and mixed m.p. 221–223°.

Epidehydrodihydroenmein (LXXII)

(i)[b] Compound IV (100 mg) was hydrogenated in AcOH (50 ml) over PtO_2 (40 mg) at room temp and press. Uptake of H_2 ceased after 1 molar equiv had been absorbed. Filtration and evaporation *in vacuo* yielded a crystalline material (100 mg), which crystallized from MeOH to give LXXII as colorless rods, m.p. 293–297°. The IR spectrum of this compound was distinguishable from that of LXXI.

$[\alpha]_D^{25}$ −91·61°; $[M]_D^{25}$ −331·63° (*c*, 1·05 in pyridine). ν_{max}^{Nujol} 3522 (hydroxyl), 1780 (γ-lactone), 1756 (five-membered ketone), 1715 cm^{-1} (δ-lactone). (Found: C, 66·58; H, 7·21. Calc. for $C_{20}H_{28}O_6$: C, 66·28; H, 7·23%.)

(ii)[b] Compound IV (100 mg) was hydrogenated in EtOH (200 ml) over Adams' catalyst (53 mg) at room temp and press. The hydrogenation was stopped when 1 molar equiv of H_2 had been absorbed.

Removal of the catalyst and evaporation of the solvent gave a crystalline solid (100 mg) which crystallized from MeOH to afford LXXII as colorless rods, m.p. 293–297°. This compound was identical with a specimen of LXXII described above.

(iii)[b] Sodium borohydride (100 mg) was added to IV (100 mg) in MeOH (150 ml) at 0°, and the mixture kept overnight at 0°. The excess reagent was destroyed with a few drops of AcOH and the mixture was concentrated *in vacuo* at 20°. After dilution with water, the mixture was extracted with AcOEt and was washed with 10% Na_2CO_3aq and then with water, dried and evaporated. The crystalline residue was recrystallized from MeOH to give LXXII as colorless rods, m.p. and mixed m.p. 293–297°.

(iv)[a] To a solution of IV (1 g) in MeOH (1 l.) was added under ice-cooling $NaBH_4$ (200 mg) in small portions and the mixture was allowed to stand overnight. After removal of the solvent under red. press. at 40°, the residue was washed with water, dried and crystallized from MeOH to give LXXII (710 mg) as prisms, m.p. 293–297°. ν_{max}^{Nujol} 3490, 1778, 1753, 1710 cm^{-1}. (Found: C, 66·32; H, 7·31. Calc. for $C_{20}H_{28}O_6$: C, 66·28; H, 7·23%.) From the mother liquor, less pure LXXII (200 mg) was obtained.

Epidehydrodihydroenmein monoacetate (LXXIII)

(i)[a] Compound LXXII was acetylated with acetic anhydride and pyridine. Recrystallization of the product from MeOH gave LXXIII as needles, m.p. 320°. The IR spectrum was not identical with that of XXXII. ν_{max}^{Nujol} 1768, 1735 cm^{-1}. (Found: C, 65·06; H, 7·13. Calc. for $C_{22}H_{28}O_7$: C, 65·33; H, 6·98%.)

(ii)[b] Compound LXXII (100 mg) in pyridine (5 ml) was treated with acetic anhydride (1 ml) at room temp overnight. The mixture was poured into water and extracted with $CHCl_3$. The extract was washed successively with 10% HClaq, 10% Na_2CO_3aq and water, dried, and evaporated. The crystalline residue (98 mg) was crystallized from MeOH–$CHCl_3$ to give LXXIII as white needles, m.p. 320°. $[\alpha]_D^{25}$ −102·68°, $[M]_D^{25}$ −414·83° (*c*, 0·97 in pyridine). (Found: C, 65·44; H, 7·05. Calc. for $C_{22}H_{28}O_7$: C, 65·34; H, 6·93%.)

Dehydrotetrahydroenmein[a]

To a solution of LXXI (300 mg) in EtOH (30 ml) was added $NaBH_4$ in small portions under ice-cooling, and the mixture was allowed to stand at room temp overnight and then heated at 60° for 1 hr. After removal of the solvent under red. press., the residue was washed with water, dried and crystallized from MeOH to give dehydrotetrahydroenmein (205 mg) as prisms, m.p. 242–243° (dec). ν_{max}^{Nujol} 3450, 1775, 1700 cm^{-1}. (Found: C, 65·83; H, 7·89. Calc. for $C_{20}H_{28}O_6$: C, 65·91; H, 7·74%.)

Acetylation of this compound with acetic anhydride and pyridine yielded the diacetate which was recrystallized from MeOH as leaflets, m.p. 256–257° (dec). ν_{max}^{Nujol} 1780, 1745, 1715 cm^{-1}. (Found: C, 64·38; H, 7·29. Calc. for $C_{24}H_{32}O_8$: C, 64·27; H, 7·19%.)

Deoxo derivative LXXIV *of* LXXI[b]

A mixture of LXXI (179 mg), ethandithiol (4 ml) and BF_3-etherate (1·5 ml) was allowed to stand for 2 days at room temp and then poured into 10% Na_2CO_3aq (50 ml). The mixture was extracted with $CHCl_3$, the extract washed with water, dried and evaporated to dryness under red. press., and

the residue (204 mg) was chromatographed in $CHCl_3$ (5 ml) on silica gel. From the first fractions eluted with $CHCl_3$, the thioketal (135 mg) of LXXI was obtained as an oil. $\nu_{max}^{CHCl_3}$ 3615, 3450 (hydroxyl), 1772 (γ-lactone), 1737 cm^{-1} (δ-lactone).

From the further $CHCl_3$ fractions, the starting material (51 mg) was recovered unchanged.

The mixture of the thioketal (135 mg) obtained above and Raney Ni (20 g) in ethanol (200 ml) was refluxed for 15 hr. The catalyst was removed by filtration and the filtrate evaporated to dryness to give a gum which was chromatographed in chloroform on silica gel. From the fraction eluted with $CHCl_3$, there was obtained a crystalline material (75 mg), which recrystallized from EtOH to give LXXIV as colorless prisms, m.p. 236–239°. ν_{max}^{KBr} 3545, 1780, 1760, 1728, 1706. $\nu_{max}^{CHCl_3}$ 3630, 3490 (hydroxyl), 1779 (γ-lactone), 1728 (δ-lactone). $\nu_{max}^{CCl_4}$ 3630 cm^{-1} (hydroxyl) (in 0·005 molar solution; Prisms; Nacl, Thickness; 10 mm). (Found: C, 68·76; H, 8·28. Calc. for $C_{30}H_{38}O_5$: C, 68·94; H, 8·10%.)

Oxidation of epidehydrodihydroenmein (LXXII)[b]

Compound LXXII (51 mg) in AcOH (8 ml) was treated with CrO_3 (30 mg) in AcOH (5 ml) and water (0·1 ml) overnight at room temp. After addition of MeOH, the mixture was concentrated under red. press., diluted with water and extracted with $CHCl_3$. The extract was washed successively with 10% Na_2CO_3aq and water, dried and evaporated to give a crystalline solid which was recrystallized from EtOH, yielding IV as needles, m.p. and mixed m.p. 221–223°.

Deoxo derivative LXXV *of* LXXII[b]

A mixture of LXXII (300 mg), ethanedithiol (4 ml) and BF_3-etherate (1·5 ml) was allowed to stand at room temp for 3 days and then poured into Na_2CO_3aq. After the extraction with $CHCl_3$, the extract was washed with water, dried and evaporated *in vacuo* to give the thioketal (420 mg) as gum. The mixture of the thioketal, Raney Ni prepared from the alloy (40 g) and EtOH (100 ml) was heated under reflux for 10 hr. After the catalyst was filtered off, the filtrate was evaporated *in vacuo* to dryness to leave an oil (295·7 mg) which was chromatographed $CHCl_3$ on silica gel. Elution with $CHCl_3$ and evaporation of the eluate afforded an oily product which crystallized on trituration with ether and was recrystallized from EtOH–ether–light petroleum, yielding LXXV as white prisms, m.p. 234–237°. ν_{max}^{KBr} 3450 (hydroxyl), 1783 (shoulder), 1748 (shoulder), 1720 cm^{-1}. $\nu_{max}^{CHCl_3}$ 3620 (hydroxyl), 3500–3450 (hydroxyl), 1783 (γ-lactone), 1732 cm^{-1} (δ-lactone). $\nu_{max}^{CCl_4}$ 3620 cm^{-1} (hydroxyl) (in 0·005 molar solution; Prisms; NaCl, Thickness; 10 mm). (Found: C, 67·07; H, 8·46. Calc. for $C_{30}H_{38}O_5$. $\frac{1}{2}H_2O$: C, 67·23; H, 8·12%.)

Compound LXXVI[b]

Compound LXXII (200 mg) was added to a solution of Na (1 g) in abs MeOH (40 ml), and the mixture heated under reflux for 4 hr. After cooling conc HCl was added until the mixture was acidic.

The NaCl which precipitated was removed by filtration and the filtrate concentrated under red. press. The resulting residue was diluted with water and extracted with $CHCl_3$. The extract was washed with 10% Na_2CO_3aq and then with water, dried and evaporated to give a crystalline solid (103 mg), which recrystallized from MeOH to afford LXXVI as white needles, m.p. 268–277°. ν_{max}^{KBr} 3480 (hydroxyl), 1781 (γ-lactone), 1756 (ester), 1715 cm^{-1} (δ-lactone). NMR in pyridine: 8·9 (doublet, J = 7 c/s, C_{16}–CH_3), 8·64 (C_4–CH_3), 8·41 (C_4–CH_3), 7·42 (C_4–H), 6·38 (—$COOCH_3$), 5·97 (doublet, J = 10 c/s, C_{16}–H), 5·27 τ (doublet, J = 10 c/s, C_{16}–H). (Found: C, 63·72; H, 7·72. Calc. for $C_{31}H_{30}O_7$: C, 63·94; H, 7·66%.)

Acetate of LXXVI[b]

Compound LXXVI (100 mg) in pyridine (4 ml) was treated with acetic anhydride (1 ml) at room temp for 2 days. The mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed successively with 10% HClaq, 10% Na_2CO_3aq and water, dried and evaporated to give a crystalline material (101 mg) which was recrystallized from EtOH, yielding the acetate of LXXVI as white scales, m.p. 207–211°. ν_{max}^{KBr} 1786–1736 (broad), 1240 cm^{-1} (acetate). NMR: 8·9 (C_4–CH_3), 8·81 (C_4–CH_3), 8·86 (doublet, J = 5·5 c/s, C_{16}–CH_3), 7·9 (OAC), 7·63 (C_5–H), 6·33 τ (—$COOCH_3$). (Found: C, 63·58; H, 7·27. Calc. for $C_{33}H_{32}O_8$: C, 63·28; H, 7·39%.)

Chromium trioxide oxidation of LXXVI[b]

Compound LXXVI (100 mg) in AcOH (7 ml) was oxidized with CrO_3 (100 mg) in AcOH (3 ml) and water (0·2 ml) at room temp overnight. The excess reagent was destroyed by adding MeOH. The mixture was concentrated *in vacuo*, diluted with water and extracted with $CHCl_3$. The extract was washed with 10% Na_2CO_3aq and then with water, and dried. After removing the $CHCl_3$, the residue was crystallized from EtOH–MeOH, giving LXII as colorless needles, m.p. 216–218°. ν_{max}^{KBr} 1788, 1760, 1726 cm^{-1}. NMR: 8·85 (doublet, J = 7 c/s, C_{16}–CH_3), 8·75 (C_4–CH_3), 8·67 (C_4–CH_3), 7·4 (C_4–H), 6·32 τ (—$COOCH_3$). (Found: C, 64·13; H, 7·36. Calc. for $C_{21}H_{28}O_7$: C, 64·27; H, 7·19%.)

Hydrolysis of LXXVI (*acid* LXXVII)[b]

A suspension of LXXVI (50 mg) in 1% KOHaq (8 ml) was heated on a water bath until the crystals disappeared completely. $AgNO_3$aq was then added to the mixture until the Ag salt of the carboxylic acid was no longer precipitated. The precipitate which formed showed absorption peaks in the IR spectrum at 1790 (γ-lactone) and 1738 cm^{-1} (δ-lactone).

10% HClaq was added to the silver salt and the mixture was extracted with AcOEt. The extract was washed with water, dried and evaporated to give a crystalline solid (45 mg) which was recrystallized from AcOEt, yielding LXXVII as prisms, m.p. 247–249°. (Found: C, 60·30; H, 7·89. Calc. for $C_{20}H_{28}O_7 \cdot H_2O$: C, 60·29; H, 7·59%.)

Phenylglyoxalate LXXVIII *of dehydrodihydroenmein* (LXXI)[a]

To a solution of LXXI (2·12 g) in pyridine (15 ml) was added dropwise phenylglyoxalyl chloride (2 g) under ice-cooling. After standing at room temp for 2 days, the mixture was concentrated under red. press. at 35°. The residue was diluted with ice-water and the resulting precipitate was collected by filtration, washed with water and dried. Recrystallization from MeOH using active charcoal yielded LXXVIII as prisms (1·45 g), m.p. 216–218°. ν_{max}^{Nujol} 1780 (γ-lactone), 1760 (five-membered ketone and ester), 1725 (δ-lactone), 1680 (benzoyl), 1595, 1500 cm^{-1} (benzene ring). (Found: C, 68·14; H, 6·24. Calc. for $C_{28}H_{30}O_8$: C, 68·00; H, 6·12%.)

Atrolactic acid from LXXVIII[a]

To the Grignard solution prepared from Mg (1·36 g), MeI (8·0 g) and tetrahydrofuran (40 ml) was added dropwise with stirring a solution of LXXVIII (1·3 g) in tetrahydrofuran (40 ml) at 22°. After 1 hr, the mixture was heated at 35–40° with stirring. The reaction mixture was decomposed with a mixture of AcOH, HCl and ice and extracted with AcOEt and then with ether. The organic layer was washed with a thiosulfate aq and water, dried and evaporated. The residue was dissolved in MeOH (700 ml) and heated under reflux with 0·583N NaOHaq (15 ml) for 4 hr. After the addition of 1N H_2SO_4 (3 ml), most of the solvent was evaporated at 40° and the residue was shaken with $CHCl_3$ and ether in order to remove neutral material (380 mg). The aqueous layer was acidified with dil HCl and extracted with ether. The ether layer was shaken with $NaHCO_3$aq and the bicarbonate solution was acidified with dil HCl and extracted with ether. Evaporation of the ether layer yielded a viscous liquid, which was chromatographed on a silica gel column. Elution with petroleum–benzin yielded crystals (120 mg), which on recrystallization from petroleum–benzin gave atrolactic acid (90 mg), m.p. 86–90°. $[\alpha]_D^{16}$ +9·2° (*c*, 0·71 in EtOH) (Lit.[38] $[\alpha]_D$ + or −37·7°). (Found: C, 61·76; H, 6·35. Calc. for $C_9H_{10}O_3 \cdot \frac{1}{2}H_2O$: C, 61·70; H, 6·33%.)

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[38] Handbuch der Organischen Chemie, Bd. 10, EI, p. 113.