UDC 547.991.02

136. **Shoichi Kanatomo**: Alkaline Decomposition of Enmein.[1]

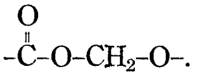
(*Pharmaceutical Faculty, University of Kanazawa**)

In the preceding paper[1] it was reported that one of the two bitter principles isolated from dry herb of *Isodon trichocarpus* has been named enmein and had a molecular formula of $C_{20}H_{26(28)}O_6$. Further, some of chemical properties of enmein were described; (1) catalytic reduction gave dihydroenmein, (2) acetylation gave diacetyl-enmein, (3) the infrared absorption spectra showed characteristic carbonyl absorptions at 5.72 and 5.88μ, and (4) enmein consumed 1 mole of alkali at room temperature and 1.8 moles at higher temerature, indicating there should be at least one lactone ring.

The object of the present work is to clarify the nature of the remaining oxygen atoms besides the four which were assigned to two hydroxyls and one lactone.

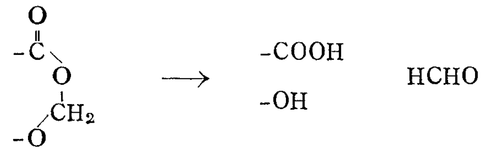
If the lactone ring existed in enmein, the same compound should be obtained by acidifying its alkali-decomposed solution, but no crystalline derivative could be obtained in spite of many attempts.

This failure showed that a part of enmein was unstable against alkali On the other hand, infrared spectrum of enmein exhibited the characteristic complicated absorptions of acetals or ketals at 8.62, 8.73, 9.02, 9.36, and 9.62 μ[2] and it was concluded that the weak point against alkali would be a kind of acetal or ketal. Actually enmein produced an aldehyde by decomposition with 1% potassium hydroxide solution and on distillation of the reaction solution over a weak flame, the distillate strongly reduced fuchsin and Tollen's reagents. From the melting point, elemental analysis, and mixed fusion with an authentic sample of formaldimedone, it was determined that the aldehyde was formaldehyde.

Thus, all the oxygen atoms in enmein seemed to be assigned; two to hydroxyls, one to a lactone, and one to a methylenedioxy group. Its infrared spectrum, however, showed two carbonyl absorptions, indicating that a ketone or aldehyde should exist in enmein, besides the lactone. Since the ketone or aldehyde occupied one oxygen atom, the lactone and the methylenedioxy should be connected, as they together occupied three oxygen atoms like  $-\overset{\overset{O}{\|}}{C}-O-CH_2-O-$. The partial formula would also be supported by the fact that enmein liberated formaldehyde very easily, like 6-nitro-1,3-benzodioxin-4-one (5-nitrosalicylic acid methylene ether ester).[3]

The remainder of enmein after removal of formaldehyde was an acid, which was obtained as a white powder after the alkali-decomposed solution was acidified. The molecular formula of the acid was $C_{19}H_{26(28)}O_6$ as expected.

These results indicated that a methylene ether ester existed in enmein and the part was hydrolyzed with alkali into formaldehyde and a hydroxy acid.



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[1] This constitutes Part II of a series entitled "Studies on Bitter Principles of *Isodon trichocarpus*" by Tetsutaro Ikeda. Part I. T. Ikeda, S. Kanatomo: Yakugaku Zasshi, **78**, 1128(1958).

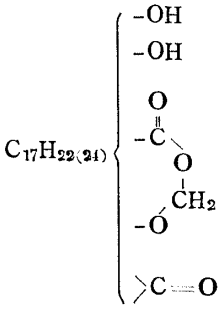
[2] E. D. Bergmann, S. Pinchas: Rec. trav. chim., **71**, 161 (1952).

[3] W. Borshe, A. D. Berkhout: Ann., **330**, 91 (1894).

As described before, the one remaining oxygen atom should be in the carbonyl group. The carbonyl is inert to all carbonyl reagents, but it should be an inert ketone, since enmein did not react with any specific aldehyde reagents.

Fortunately, an oxime of diacetylenmein was obtained, though in a very poor yield, after many experiments and direct proof of the presence of a ketone was established.

Thus, the oxygen atoms in enmein were all accounted for and enmein could be represented by the following partial formula:



The new methylene ether ester of hydroxy acid is very interesting since this is the first discovery of the group in natural products and even in synthesized substances, except in some aromatic compounds.

The author is greatly indebted to Prof. T. Ikeda and Dr. T. Kosuge of the University of Kanazawa for valuable suggestion and encouragements, and also expresses his thanks to Mr. Y. Matsui of Shionogi & Co. for the measurement of infrared spectra. The microanalyses were carried out by Mr. Y. Itatani to whom he is also indebted.

Experimental

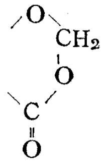
All temperatures are uncorrected. Infrared absorption spectra were measured by Perkin-Elmer Model 12 single-beam spectrophotometer as a Nujol mull.

Hydrolysis of Enmein—i) Identification of Formaldehyde: In a round-bottomed flask connected with a condenser, a mixture of 1.0 g. of enmein and 100 cc. of 1% KOH was heated over a weak flame. The gas evolved was collected in a vessel containing saturated aqueous solution of dimedone. The heating was continued until the residual solution measured around 10 cc. The dimedone (0.040 g.) was obtained as colorless crystals which recrystallized from dil. EtOH to colorless needles, m.p. 187~189°. *Anal.* Calcd. for $C_{17}H_{24}O_4$ (Formaldimedone): C, 69.93; H, 8.27. Found: C, 69.89, 69.79; H, 8.19, 8.22. The dimedone was identified with authentic sample of formaldimedone (m.p. 188~189°) by mixed fusion.

ii) Separation of the Acid: A mixture of 10.0 g. of enmein and 300 cc. of 5% KOH soln. was allowed to stand overnight and submitted to steam distillation for 6 hrs. to remove HCHO produced. After cool, the mixture was acidified with dil. H_2SO_4, the precipitate was collected, and washed several times with water. Yield, 7.5 g. The acid was dissolved in dil. $NaHCO_3$ soln. and reprecipitated with dil. H_2SO_4. Several recrystallizations from AcOEt gave yellowish crystals, m.p. 293~294° (decomp.). *Anal.* Calcd. for $C_{19}H_{26(28)}O_6$: C, 65.12 (64.75); H, 7.48 (8.01). Found: C, 65.47, 65.33; H, 7.60, 7.56.

Diacetyl-enmein Oxime—A mixture of 4.5 g. of diacetyl-enmein, 8 g. of hydroxylamine hydrochloride, 14.0 g. of AcONa, 100 cc. of EtOH, and 50 cc. of water was heated on a steam bath for 5 hrs. After the solvents were removed under diminished pressure, the residue was washed with water and 1.5 g of a crude product was obtained. The product was chromatographed over alumina column with AcOEt as a solvent. The column was eluted with MeOH and, after removal of MeOH, the residue was rechromatographed over silica gel with AcOEt. The part which colored green to $FeCl_3$ in $CHCl_3$ was recrystallized from $CHCl_3$-benzene mixture to pale yellowish crystals, m.p. 300~310° (decomp.). The yield was only 100 mg. Microanalysis was carried out after drying over P_2O_5 and paraffin at 150° under diminished pressure. *Anal.* Calcd. for $C_{21}H_{31(33)}O_8N$: C, 62.45 (62.19); H, 6.77 (7.18); N, 3.04 (3.02). Found: C, 62.62; H, 7.09; N, 2.67.

Summary

The nature of all six oxygen atoms in enmein, $C_{20}H_{26(28)}O_6$, were clarified as two of hydroxyls, one of inert ketone, and one of methylene ether ester of hydroxy acid. The discovery of a new group, methylene ether ester of hydroxy acid  seemed of great interest.

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137. Takeshi Oda and Hiroko Kamiya: On the Complex Lipid, Cerebrine Phosphate, of Yeast.

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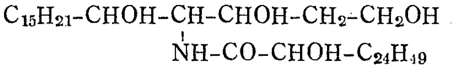
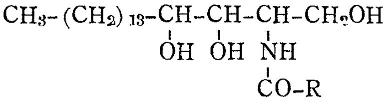
Reindel and others[1,2] made detailed studies on the complex lipid of yeast, cerebrine, and proposed the following structure for it.



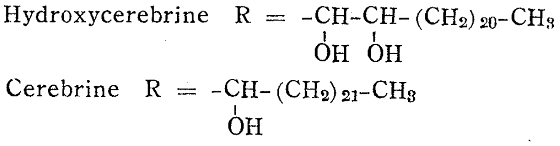
$$C_{15}H_{21}\text{-CHOH-}\underset{\text{NH-CO-CHOH-}C_{24}H_{49}}{\text{CH}}\text{-CHOH-}CH_2\text{-}CH_2OH \qquad (C_{46}H_{93}O_5N)$$

On the other hand, Ruppol[3] isolated a complex lipid corresponding to the formula $C_{46}H_{91}O_4N$ from beer yeast and reported that its hydrolysis afforded 2-hydroxy acid of C_{28} and sphingosine, but validity of the latter is questionable.

One of the writers (T.O.) obtained cerebrine from the mycelium of surface-cultivated penicillin-producing fungus and corrected Reindel's structural formula, establishing its chemical structure to be as follows:



$$CH_3\text{-}(CH_2)_{13}\text{-}\underset{OH}{CH}\text{-}\underset{OH}{CH}\text{-}\underset{\underset{\text{CO-R}}{NH}}{CH}\text{-}CH_2OH$$



$$\text{Hydroxycerebrine}\quad R = \text{-}\underset{OH}{CH}\text{-}\underset{OH}{CH}\text{-}(CH_2)_{20}\text{-}CH_3$$

$$\text{Cerebrine}\quad R = \text{-}\underset{OH}{CH}\text{-}(CH_2)_{21}\text{-}CH_3$$

Carter and others[5] isolated a long-chain base by hydrolysis of the complex lipid fraction, which had heretofore been called inositol lipid, from soybean and corn (*Zea Mays* L.) and proved that it was identical with the cerebrine base obtained from the above-mentioned *Penicillium* sp. They named this phytosphingosine, as against sphingosine isolated from animals. Cerebrine had been isolated from the mycelium of surface-cultivated penicillin-producing fungus and from *Aspergillus sydowi*,[6] as a product of autolysis, and yeast cere-

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* Oshika, Shizuoka (小田 武, 神谷弘子).

1) F. Reindel: Ann., **480**, 76(1930).

2) F. Reindel, A. Weickmann, S. Picard, K. Luber, P. Tulula: *Ibid.*, **544**, 116(1940).

3) E. Ruppol: Bull. soc. chim. biol., **19**, 1164(1937).

4) T. Oda: Yakugaku Zasshi, **72**, 136(1952).

5) H. E. Carter, W. D. Celmer, M. L. Willaim, L. M. Catherine, H. H. Tomizawa: J. Biol. Chem., **206**, 613(1954).

6) N. Bohonos, W. H. Peterson: *Ibid*, **149**, 295(1943).