mutiplet signals (5.6 τ) and low field multiplet signals (5.45 τ) are α- and β-hydrogen of rings for substituents respectively, and acetyl singlet signal was shown at 7.87 τ.

Two isomers of acetylbiferrocenyl, m.p. 158° and 142~144°, were considered by means of infrared, ultraviolet, nuclear magnetic resonance spectra, and chromatographic behaviour, which structures were assumed 3-acetyl-1-biferrocenyl (III) and 1′-acetyl-1-biferrocenyl (IV) respectively.

From above results, it is concluded that electrophilic substitution reactivity of biferrocenyl decrease due to intraannular electronic effects of ferrocenyl group. And also this main reaction occur in heteroannular ring at 1′-position, and reactivity homoannular ring deactivates the substituted ferrocene ring towards acylation.

Tokyo Research Institute
Yawata Iron & Steel Co., Ltd.
1618 Ida, Kawasaki, Kanagawa-Ken.

Koji Yamakawa (山川浩司)
Naoharu Ishibashi (石橋修治)
Kiichi Arakawa (荒川基一)

Received October 11, 1963

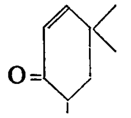
[Chem. Pharm. Bull. 12 (1) 121 ~ 124]

UDC 615.779.932 : 547.92

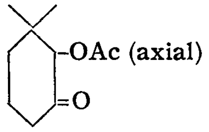
The Structure of Helvolic Acid

Helvolic acid, a steroidal antibiotic, has been the subject of fairly extensive chemical and biological studies,[1] from the time it was first isolated in an impure state from the culture filtrate of *Aspergillus fumigatus.*[2] From the chemical and spectral studies of this compound and its derivatives, Allinger proved the following partial structures exist in helvolic acid.*[1]

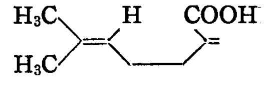
1. 6-Membered α,β-unsaturated ketone possessing a secondary α'-Methyl group but no γ-hydrogen atom :



2. 6-Membered α-axial-acetoxyketone having no β-hydrogen atom :



3. 2-Alkylidene-6-methyl-5-heptenoic acid side chain :



By combining these partial structures and predicting*[2] that an α-acetoxy ketone would be in ring C and that another acetoxy group at C$_{16}$-position, formura (I) was proposed as a tentative structure of this antibiotic.

This paper reports the isolation of helvolic acid from the culture broths of *Cephalosporium caerulens* and *Emericellopsis terricola* van BEYMA and also proposes a revised

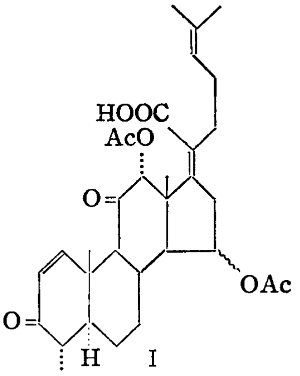
---

*[1] This is in complete agreement with our experimental data.

*[2] These predictions are quite contrary to the data presented in this communication.

1) *a*) N.L. Allinger, J.L. Coke : J. Org. Chem., **26**, 4522 (1961). *b*) N.L. Allinger : J. Org. Chem., **21**, 1180 (1956). *c*) For the summary of earlier literature and references see D.J. Cram, N.L. Allinger : J. Am. Chem. Soc., **78**, 5278 (1956).

2) S.A. Wacksman, E.S. Horning, E.L. Spencer : J. Bact., **45**, 233 (1943).



I

Chart 1.

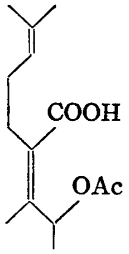


Chart 2.

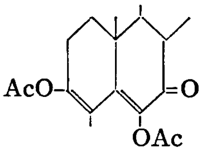


Chart 3.

formula (II)[*3] for the structure of helvolic acid.

Both butyl acetate extracts of the culture filtrates of *Cephalosporium caerulens* and *Emericellopsis terricola* afforded white needles, m.p. 215°(methanol), $[\alpha]_D^{25}$: −121°($CHCl_3$), *Anal.* Calcd. for $C_{33}H_{44}O_8$: C, 69.69; H, 7.80. Found : C, 69.47; H, 7.76, UV λ_{max}^{EtOH} mμ (log ε) : 231 (4.24). The yeilds were 1000 γ/ml. and 40 γ/ml., respectively. Treatment with diazomethane gave a methyl ester (III), m.p. 257°(methanol), $[\alpha]_D^{25}$: −140°($CHCl_3$), *Anal.* Calcd. for $C_{34}H_{46}O_8$: C, 70.08; H, 7.96. Found : C, 69.73; H, 7.91., UV λ_{max}^{EtOH} mμ (log ε) : 231 (4.24), IR ν_{max}^{Nujol} cm^{-1} : 1745 (broad : C_7-, C_{16}-OAc), 1718 (6-keto, α,β-unsaturated ester), 1675, 1655 (Δ^1-3-one), 1255, 1238, 1210 $(-C-O-\overset{\overset{O}{\|}}{C}-)$. This was identified with an authentic specimen of methyl helvolate[*4] by comparison of the infrared spectra and mixed melting point test. The nuclear magnetic resonance spectra of III showed the absorptions of three tertiary methyl groups (8.55, 8.82 and 9.08 τ), isopropylidene methyls (8.30, 8.38 τ), one secondary methyl (8.72 τ, J : 6.5 c.p.s.), two acetyl methyls (7.90, 8.05 τ), α- and β-protons of α,β-unsaturated ketone (4.17, 2.70 τ, J : 11.4 c.p.s.), α-proton of α-acetoxy ketone (4.78 τ singlet) and a proton of a type $-CH_2-\overset{\overset{OAc}{|}}{\underline{CH}}-C=O$ (4.17 τ, Js could not be determined because methyl signal overlapping.[*5]). This indicates that an additional tertiary of the group should be contained in helvolic acid as compared with formula (I).

Reductive deacetoxylation of helvolic acid with zinc and acetic acid afforded a dihydrodesacetoxy derivative (IV), m.p. 205°, *Anal.* Calcd. for $C_{31}H_{44}O_6$: C, 72.62; H, 8.65. Found : C, 72.60; H, 8.67, UV λ_{max}^{EtOH} mμ (log ε) : 219 (3.95), IR ν_{max}^{Nujol} cm^{-1} : 1742 (C_{16}-OAc), 1710 (3-, 6-keto), NMR : C_{16}-H (4.15 τ, quartet, J : ca 8.0 and 2.0 c.p.s.). Hydrolysis of IV with 0.3*N* aqueous sodium hydroxide solution, followed by acidification with dilute hydrochloric acid, yielded an unsaturated lactone (V), m.p. 162~163°, *Anal.* Calcd. for $C_{29}H_{40}O_4$: C, 76.95; H, 8.91. Found : C, 76.91; H, 8.87, UV : λ_{max}^{EtOH} 221.5 mμ (log ε 3.954), IR : ν_{max}^{Nujol} 1750 cm^{-1} (5-membered α,β-unsaturated lactone), no absorption corresponding to OH, COOH or $CH_3\overset{\overset{O}{\|}}{C}-O-$. Catalytic hydrogenation of V with platinum oxide in acetic acid, with three mole hydrogen uptake, gave a saturated lactone (VI), amorphous solid, IR : ν_{max}^{Nujol} 1763 cm^{-1} (γ-lactone). The nuclear magnetic resonance spectra of both compounds also show the presence of the additional tertiary methyl group.

To confirm the molecular weight of V, the mass spectra was determined and m/e 453 (M+1) and 452 (M) peaks were observed. Therefore the correct empirical formula

---

[*3] This represents not a configurational but a skeletal structure.

[*4] This was kindly supplied by Professor N. L. Allinger of Wayne State University, U. S. A.

[*5] The quartet signal of this type proton was observed in the case of 1,2-dihydro derivative such as IV.

of V and helvolic acid are $C_{29}H_{40}O_4$ and $C_{33}H_{44}O_8$,[*6],[*7] respectively.

Although the 15-acetoxy group was formerly proposed on the basis of the pyrolytic formation of a homocyclic conjugated diene involving decarboxylation and elimination of an acetoxy group,[1a] the formation of lactone (V) clearly indicates that the partial structure shown in Chart 2 should exist in helvolic acid if the original orientation of the α,β-unsaturated carboxylic acid is retained during hydrolysis of IV.[*8]

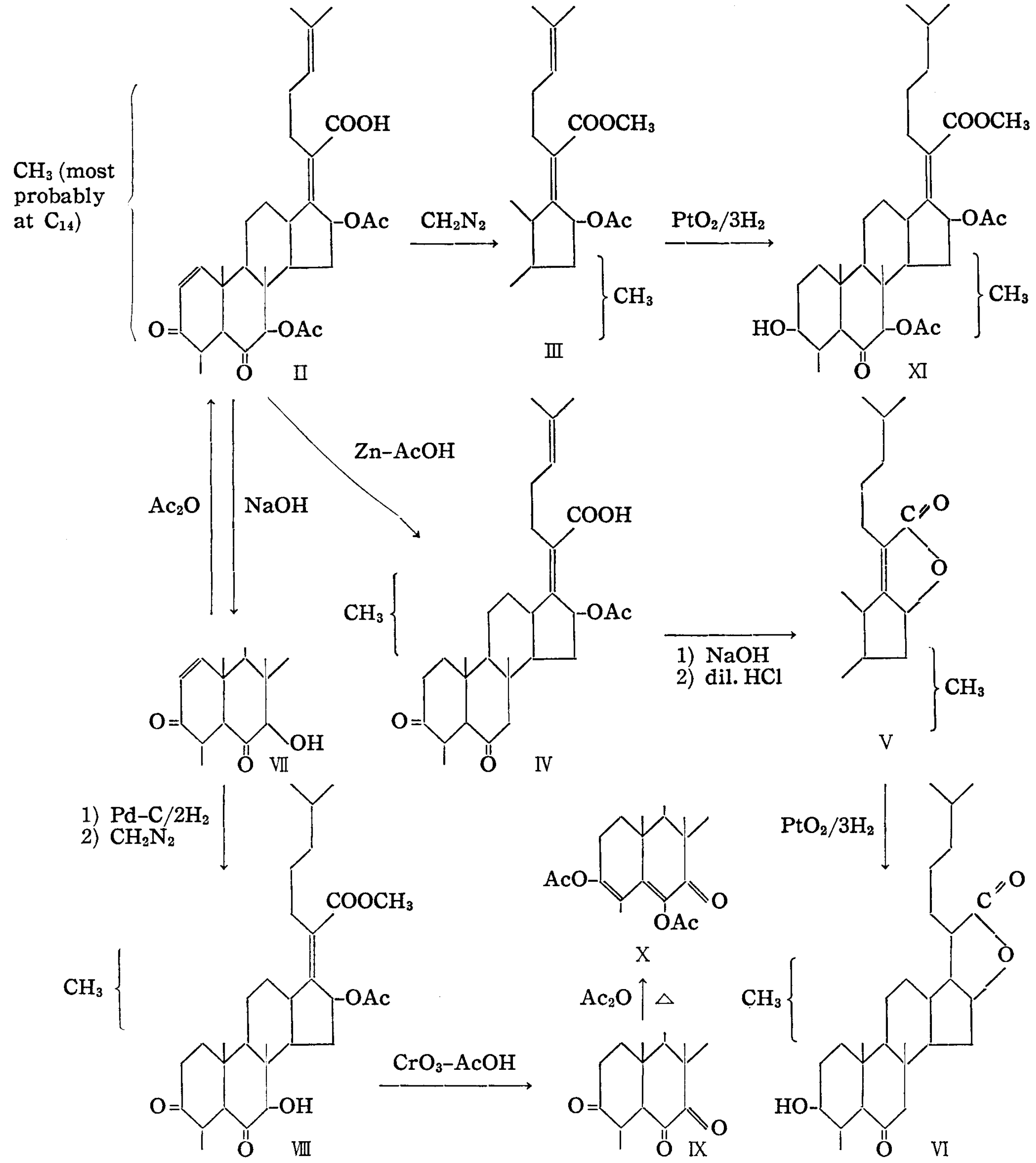


Chart 4.

[*6] All analytical data of helvolic acid and its derivatives also corresponded to this empirical formula.

[*7] Recently it was reported that a mass spectrum of helvolic acid showed m/e 508 (M-AcOH) peak: J. F. Lynch, J. M. Wilson, H. Budzikiwicz, C. Djerassi: Experientia, **19**, 211 (1963). This also requires the empirical formula $C_{33}H_{44}O_8$.

[*8] It can be reasonably assumed that there is no epimerization at the stage of hydrolysis but the inversion of C_{16}-O bond takes place during the lactonization by a S_{N2} type attack by the carboxyl group. Details will be discussed elsewhere.

Regarding to the position of α-acetoxy ketone group, it was proposed that this should be in ring C not in ring B since methyl hexahydrohelvolate (XI) exhibits a strongly negative Cotton effect.[1a] However this proposal seems unacceptable because 11α-, 11β-acetoxy-12-oxo-, 12α- and 12β-acetoxy-11-oxo-5β-steroids all show a positive Cotton effect,[3] whereas 6- or 7-oxo-5α-steroids show negative Cotton effect.[4] To elucidate the position of this group methyl tetrahydrohelvolinate (VIII) was oxidized to a triketone (IX), m.p. 191~193°, *Anal.* Calcd. for $C_{32}H_{46}O_7$: C, 70.82; H, 8.54. Found : C, 7.47; H, 8.58, which was converted into an enol acetate (X), m.p. 103~104°, *Anal.* Calcd. for $C_{36}H_{50}O_9$: C, 68.98; H, 8.04. Found : C, 68.79; H, 7.99. The spectral data of this enol acetate, UV λ_{max}^{EtOH} mμ (log ε) : 219 (4.05), 287.5 (4.16), IR ν_{max}^{Nujol} cm^{-1} : 1768 (C_6-OAc), 1750~1720, (C_3-, C_{16}-OAc, α,β-unsaturated ester) 1683, 1655, 1615 ($\Delta^{3,5}$-7-one),[5] NMR : 7.79 (two acetyl methyls), 8.03 (one acetyl methyl), 8.15 τ (C_4-methyl : singlet) and no absorption corresponding to a vinyl proton. strongly suggests the presence of the chromophor shown in Chart 3[5,6] Consequently the α-acetoxy ketone group of helvolic acid is most probably located in ring B and 7-acetoxy-6-oxo structure is preferable since in the nuclear magnetic resonance spectrum of III the signal of α-proton of this group is singlet.

From the similarity of helvolic acid to cephalosporin P_1[6] and fusidic acid,[7] it seems quite reasonable that it also possesses a fusidic acid type skeleton. Therefore summarizing the available data, formula (II) is proposed for the structure of helvolic acid and the reactions described in this communication are shown in Chart 4. Further studies on the structure and stereochemistry of this compound are now under investigation.

The Authors are grateful to Dr. K. Takeda of Research Laboratory, Shionogi & Co., Ltd., Mr., M. Matsui of Research Laboratories, Sankyo Co., Ltd., Professor T. Hoshino of Basic Research Laboratories, Toyo Rayon Co., Ltd., for NMR spectra and to Drs. T. Noda, Y. Nakajima and H. Sato of Naka work, Hitachi Products, Ltd. for mass spectra. Their thanks are also due to Misses H. Yamanouchi, K. Hayashi and Mr. T. Tsuruta of the Institute of Applied Microbiology, Tokyo University, for microanalyses and IR spectra.

The Institute of Applied Microbiology
University of Tokyo
Mukogaoka-Yayoicho, Bunkyo-ku, Tokyo.

Shigenobu Okuda (奥田重信)
Shigeo Iwasaki (岩崎成夫)
Kyosuke Tsuda (津田恭介)

Kitasato Institute
Shibashiroganesanko-cho
Minato-ku, Tokyo.

Yoshimoto Sano (佐野敬元)
Toju Hata (秦藤樹)

National Institute of Hygienic Sciences
Tamagawa-Yoga, Setagaya-ku, Tokyo.

Shun-ichi Udagawa (宇田川俊一)

Nippon Kayaku Co., Ltd.
Shimo-sanchome, Kita-ku, Tokyo.

Yuya Nakayama (中山勇彌)
Hiroshi Yamaguchi (山口宏)

Received October 28, 1963

---

3) C. Djerassi, O. Halperm, V. Halpern, O. Schindler, Ch. Tamm : Helv. Chim. Acta., **41**, 250 (1958).
4) C. Djerassi, W. Closson, A.E. Lipman : J. Am. Chem. Soc., **78**, 3163 (1956).
5) K. Yasuda : Chem. Pharm. Bull., **11**, 1167 (1963).
6) *a*) B.M. Baird, T.G. Halsall, E.R.H. Jones, G. Lowe : Proc. Chem. Soc., **1961**, 257. *b*) T.G. Halsall, E.R.H. Jones, G. Lowe : *Ibid.*, **1963**, 16.
7) W.O. Godtfredsen, S. Vangedal : Tetrahedron, **18**, 1029 (1962).