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3-Oxo-16β-acetoxyfusida-1, 17(20)[16, 21-cis], 24-trien-21-oic Acid の単離と構造研究*1

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Isolation and Structural Elucidation of 3-Oxo-16β-acetoxyfusida-1, 17(20)[16,21-cis],24-trien-21-oic Acid*1

Yoshihiro Sato

This communication is concerned with the isolation of 3-oxo-16β-acetoxyfusida-1,17-(20)[16,21-cis],24-trien-21-oic acid from the culture broth of *Cephalosporium caerulens* and elucidation of its structure (IIa).

After removal of a great part of helvolic acid (Ia)[1] from the extracted metabolites mixture by recrystallization, a careful separation of the mother liquor by silica gel column chromatography gave a crystalline acid (yield: 1.57 mg/1000 ml) eluted just before the Ia fraction. The free acid (IIa): m.p. 204°, $C_{31}H_{44}O_5$*2, $[\alpha]_D^{25}$ +34.7°, UV: 237 (4.20), IR: 3260, 1735, 1717, 1677, 1250, 1028, 1018. The methyl ester (IIb): m.p. 119°, $C_{32}H_{46}O_5$, M^+ 510, $[\alpha]_D^{25}$ +25.8°, IR: 1735, 1725, 1678, 1250, 1165, 1030, 1012. The UV and IR spectra indicate the presence of an α,β-unsaturated ketone and an α,β-unsaturated carboxylic acid group in this compound. The NMR spectral data of the ester (IIb) are quite similar to those of methyl helvolate (Ib) and methyl 7-desacetoxyhelvolate (Ic)[2] as shown in Table I. These spectral data, molecular formula $C_{31}H_{44}O_5$, and the origin of this metabolite suggest that this compound should be one of the precursors of Ia, which most probably possesses a fusidane structure containing Δ^1-3-ketone, 4α-CH_3, 16β-OAc, $\Delta^{17(20)}$[16,21-cis]-21-carboxylic acid and $>C=C_{25}<^{CH_3}_{CH_3}$.

To prove the above assumption, this acid was labeled with ^3H by the Wilzbach method[3] and fed to the culture of *C. caerulens*. Ten mg of the acid (4.1×10^5 dpm/mg) were administered to the culture (100 ml) preincubated for 3 days and worked up as usual after a further shaking for 3 days. The Ia extracted was purified to give a constant specific activity by a combination of column chromatography and recrystallization. The yield and specific activity were 12 mg and 7.3×10^4 dpm/mg. This high incorporation (21%) demonstrated the identity of the framework of this acid and that of Ia, and consequently this compound must be IIa. Our attempts in the past few years to show the chemical interrelation be-

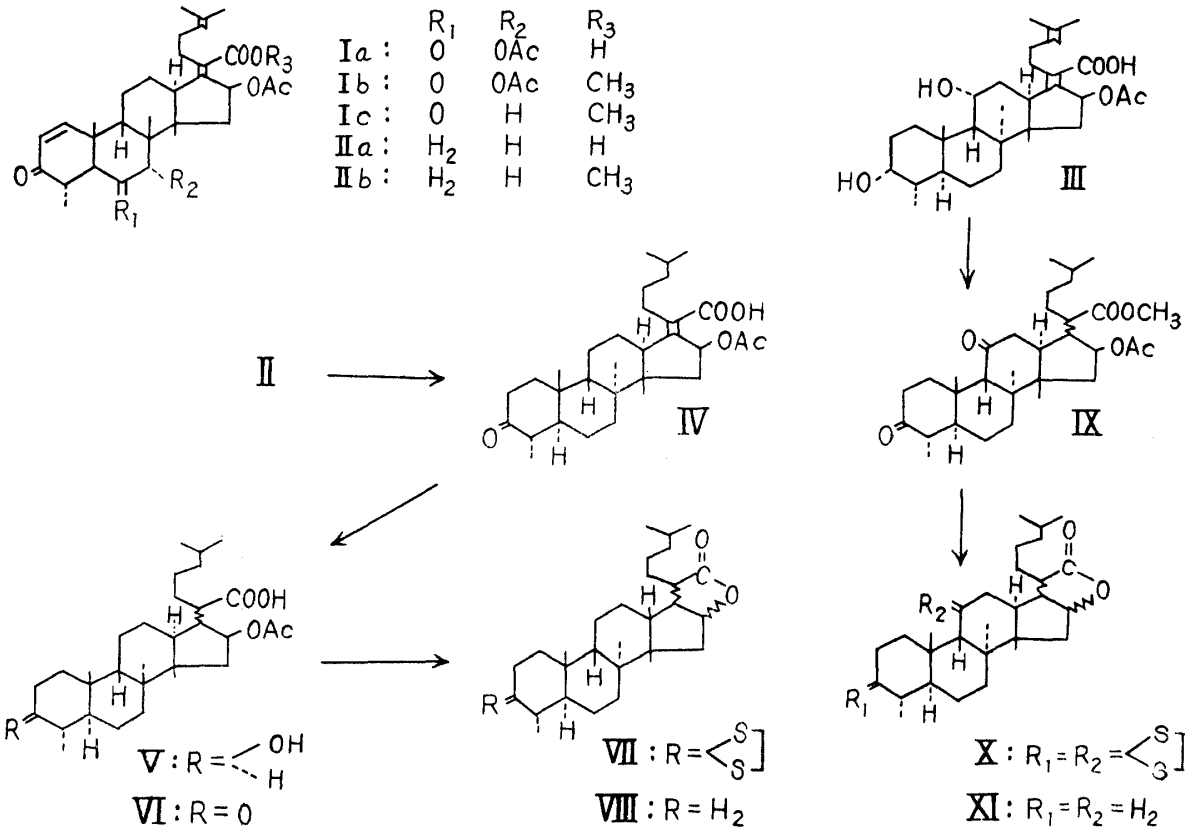
*1 本報告は S. Okuda, Y. Sato, T. Hattori, M. Wakabayashi, *Tetrahedron letters*, 4847 (1968) に発表.

*2 The compounds whose molecular formula are cited gave satisfactory analytical data. Unless otherwise stated, the NMR (δ), UV (mμ), IR spectra (cm^{-1}) and $[\alpha]_D$ were taken in $CDCl_3$, nujol, EtOH and $CHCl_3$, respectively.

1) S. Okuda, S. Iwasaki, K. Tsuda, Y. Sano, T. Hata, S. Udagawa, Y. Nakayama, and H. Yamaguchi, *Chem. Pharm. Bull. (Tokyo)*, **12**, 121 (1964).

2) S. Okuda, Y. Nakayama, and K. Tsuda, *ibid.*, **14**, 436 (1966).

3) K.E. Wilzbach, *J. Am. Chem. Soc.*, **79**, 1013 (1957).



tween Ia and fusidic acid (III)[4] were all unsuccessful because of great difficulty in removing the oxygen functional groups in the B–ring of Ia. If the acid in question is really IIa possessing no oxygen group in the B–ring, this compound may be related to the 11–deshydroxy derivative of III without great difficulty. This is attractive since it also suggests the possibility of relating Ia to III*3 by a combination of chemical and biological techniques.

Catalytic hydrogenation of this acid first over 10% Pd/C in EtOH and then over PtO_2 in AcOH afforded a tetrahydro derivative (IV), m.p. 206°, $C_{31}H_{48}O_5$, and an octahydro derivative (V)*4, m.p. 138°, $C_{31}H_{52}O_5$. Oxidation of V with CrO_3 in AcOH furnished an oily 3–ketone (VI), $C_{31}H_{50}O_5$, which was heated with a mixture of ethandithiol and BF_3–etherate at 80°. Thioketalization and lactonization afforded VII, m.p. 224°, $C_{31}H_{50}O_2S_2$, IR: 1764. The treatment of VII with Raney Ni (W–4) in refluxing EtOH, followed by catalytic hydrogenation over PtO_2 in AcOH for the reduction of a minor olefinic product, furnished a lactone (VIII)*5, m.p. 149°, $C_{29}H_{48}O_2$, M^+ 428, $[\alpha]_D^{25}$ +40.0°, NMR: C_{16}–H

*3 Recently the group of Leo Pharmaceutical Products has succeeded in interrelating III and Ia by an elegant combination of microbial and chemical techniques (W. von Daehne, and H. Lorck, Abstracts, 5th International Symposium on the Chemistry of Natural Products, London, 1968, p. 337—338).

*4 The hydrogenation of $\Delta^{17(20)}$ probably takes place from the β–side as in the case of III[4].

4) W. O. Godtfredsen, W. von Daehne, S. Vangedal, A. Marquet, D. Arigoni, and A. Melera, *Tetrahedron*, **21**, 3505 (1965) and the references cited there.

*5 The stereochemistry of the lactones have not been studied.

TABLE I

	Ib	Ic	Methyl ester of the acid (IIb)	
C_1, C_2–H	7.30 (d, J=10.0) 5.83 (d, J=10.0)	7.30 (d, J=10.0) 5.84 (d, J=10.0)	O=C–C=C–C– H H	7.35 (d, J=10.0) 5.83 (d, J=10.0)
$C_{16\beta}$–H	5.83 (d, J=8.0)	5.78 (d, J=8.0)	>C(H)–O–C(=O)–CH_3	5.81 (d, J=8.0)
C_7–H	5.22 (s)	——	——	——
C_{24}–H	5.11 (m)	5.06 (m)	olefinic–H	5.09 (m)
$C_{21}OOCH_3$	3.63 (s)	3.62 (s)	–$COOCH_3$	3.62 (s)
$C_{7\alpha}$–C(=O)–CH_3	2.10 (s)	——	——	——
$C_{16\beta}$–C(=O)–CH_3	1.95 (s)	1.94 (s)	–O–C(=O)–CH_3	1.98 (s)
H>C=C_{25}<CH_3 CH_3	1.70 (s) 1.62 (s)	1.66 (s) 1.58 (s)	>C=C<CH_3 CH_3	1.68 (s) 1.60 (s)
–$C_{4\alpha}$(H)–CH_3	1.28 (d, J=6.5)	1.14 (d, J=ca. 6)	–C(H)–CH_3	1.15 (d, J=7.0)
–C–CH_3	1.45 (s) 1.18 (s) 0.92 (s)	1.30 (s) 1.09 (s) 0.88 (s)	–C–CH_3	1.18 (s) 1.02 (s) 0.99 (s)

(4.79, m), $(CH_3)_2$·CH– (0.92 (3H), 0.85 (3H)), CH_3–C– (1.10 (3H), 0.85 (6H)), C_4–CH_3 (overlapping the other methyl signals), IR(KBr): 1764 (5 membered lactone).

On the other hand, the methyl ester (IX) of a diketoacid, m.p. 98°, $C_{32}H_{50}O_6$, derived from III[4], was heated with a mixture of ethandithiol and BF_3–etherate to afford a dithioketal lactone (X)*[5], m.p. 293.5—294.5°, $C_{33}H_{52}O_2S_4$, IR: 1770 (5 membered lactone). Desulfurization of X with Raney Ni (W–4) and subsequent hydrogenation over PtO_2 gave white crystals. Column chromatography on $AgNO_3$–silica gel and recrystallization furnished a pure saturated lactone (XI), m.p. 149°, $[\alpha]_D^{25}$+37.0°, which is completely identical with VIII derived from the acid (IIa), in all respects, i.e., IR., NMR, mass spectrum, optical rotation and the mixed melting point test.

Thus the chemical interrelation between the acid (IIa) and fusidic acid (III) has established the identity of the framework of the two acids. This and the above microbial conversion of the acid into helvolic acid (Ia) clearly demonstrate that this acid is 3–oxo–16β–acetoxyfusida–1,17(20)[16,21–cis],24–trien–21–oic acid (IIa) and also prove the correctness of the proposed structure Ia of helvolic acid[5].

5) S. Okuda, S. Iwasaki, M.I. Sair, Y. Machida, A. Inoue, and K. Tsuda, *Tetrahedron Letters*, **24**, 2295 (1967).

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