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3 $\beta$ -Hydroxy-4 $\beta$ -hydroxymethylfusida-17(20)[16, 21-cis], 24-diene  
の単離

佐藤 良博

Isolation of 3 $\beta$ -Hydroxy-4 $\beta$ -hydroxymethylfusida-17(20)-  
[16,21-cis],24-diene\*<sup>1</sup>  
Yoshihiro Sato

Recently the intact incorporation of the squalene chain into the fusidane skeleton was demonstrated and 3 $\beta$ -hydroxy-4 $\beta$ -methylfusida-17(20)[16,21-cis],24-diene (II) was proposed as the precursor of fusidic acid (III)<sup>1)</sup>. In this communication we wish to report the isolation of one of the precursors of helvolic acid (IV) whose structure is assigned to be 3 $\beta$ -hydroxy-4 $\beta$ -hydroxymethylfusida-17(20)[16,20-cis],24-diene (V).

After a great part of IV of the metabolites mixture extracted from the mycelia of *Cephalosporium caerulens* was removed by recrystallization, the components of the mother liquor were chromatographed on silical gel column. Recrystallization of the eluate between ergosterol and IV afforded a diol\*<sup>2</sup> (V), m.p. 143°, C<sub>30</sub>H<sub>50</sub>O<sub>2</sub>\*<sup>3</sup> M<sup>+</sup> 442, [ $\alpha$ ]<sub>D</sub><sup>20</sup> +19.1°, IR (CCl<sub>4</sub>, C=0.0026 M. the calibration standard: indene) 3628±1 (sharp, nonbonded OH), 3567±1 cm<sup>-1</sup> (broad, bonded OH), diacetate (VI), m.p. 96°, C<sub>34</sub>H<sub>54</sub>O<sub>4</sub>, M<sup>+</sup> 526, [ $\alpha$ ]<sub>D</sub><sup>20</sup> +32.6°.

As shown in Table I, the NMR spectra indicate the existence of three methyls on the double bond, four tertiary methyls, and the partial structures >CH-OR and  $\begin{array}{c} | \\ -\text{C}-\text{CH}_2\text{OR} \\ | \end{array}$  (R=H or Ac).

These NMR spectral data, the molecular formula, and the origin of this diol strongly suggested that this must be one of the precursors of IV in which a secondary and a primary hydroxy groups are most probably located at C<sub>3</sub>-position and at one of the two C<sub>4</sub>-methyls. To confirm this assumption, 2.0 mg of the diol <sup>3</sup>H-labeled by the Wilzbach method<sup>2)</sup> (2.61×10<sup>7</sup> dpm/mg) was fed into a culture of *C. caerulens* (100 ml), preincubated for 2 days and continued cultivation for further 5 days. The usual work up followed by silica gel column chromatography and one recrystallization furnished IV, m.p. 214—5°, 14.45 mg. After dilution with 73.20 mg of cold IV, seven recrystallizations gave the specific activity 1.03×10<sup>4</sup> dpm/mg. This incorporation (1.71%) demonstrated that this compound is one of the intermediates in the main biogenetic path of IV and consequently the

\*1) 本報告は S. Okuda, Y. Sato, T. Hattori, H. Igarashi, T. Tsuchiya, N. Wasada, *Tetrahedron letters*, 4769 (1968) に発表.

\*2) This diol was kindly identified with the sample isolated from the culture of *Fusidium coccineum* (a private communication from Dr. W.O. Godtfredsen).

\*3) The compound whose molecular formula is cited gave satisfactory analytical data. Unless otherwise stated, NMR ( $\delta$ ) and [ $\alpha$ ]<sub>D</sub> were taken in CDCl<sub>3</sub> and CHCl<sub>3</sub> respectively.

1) W.O. Godtfredsen, H. Lorck, E.E. van Tamelen, J.D. Willett, R.B. Clayton, *J. Am. Chem. Soc.*, 90, 208 (1968).

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

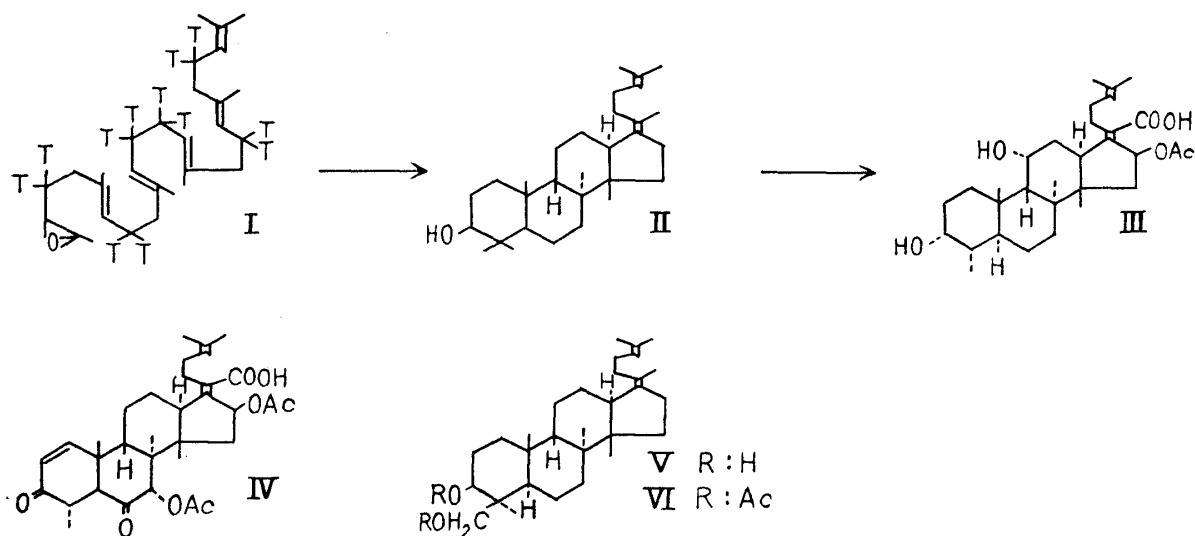


TABLE I

	$\begin{array}{c} \text{OR} \\   \\ -\text{C}-\text{H} \end{array}$	$\text{H} > \text{C} = \text{C} <$	$\begin{array}{c}   \\ -\text{C}-\text{CH}_2\text{OR} \\   \end{array}$	$> \text{C} = \text{C} < \text{CH}_3$	$\begin{array}{c}   \\ -\text{C}-\text{CH}_3 \\   \end{array}$	$\begin{array}{c} \text{O} \\    \\ -\text{O}-\text{C}-\text{CH}_3 \end{array}$
V (R=H)	3.43 <sup>a)</sup> diffused t. J=ca 8	5.10 (m)	3.26 slightly diffused d, J=11.0 <sup>b)</sup> 4.21 d, J=11.0 <sup>b)</sup>	1.57 1.58 1.67	0.73 0.88 1.11 1.20	
VI (R=Ac)	4.60 diffused t. J=ca 8	5.10 (m)	4.16 d, J=12.0 4.29 d, J=12.0	1.59 1.60 1.67	0.76 0.97 1.02 1.14	2.03 2.05

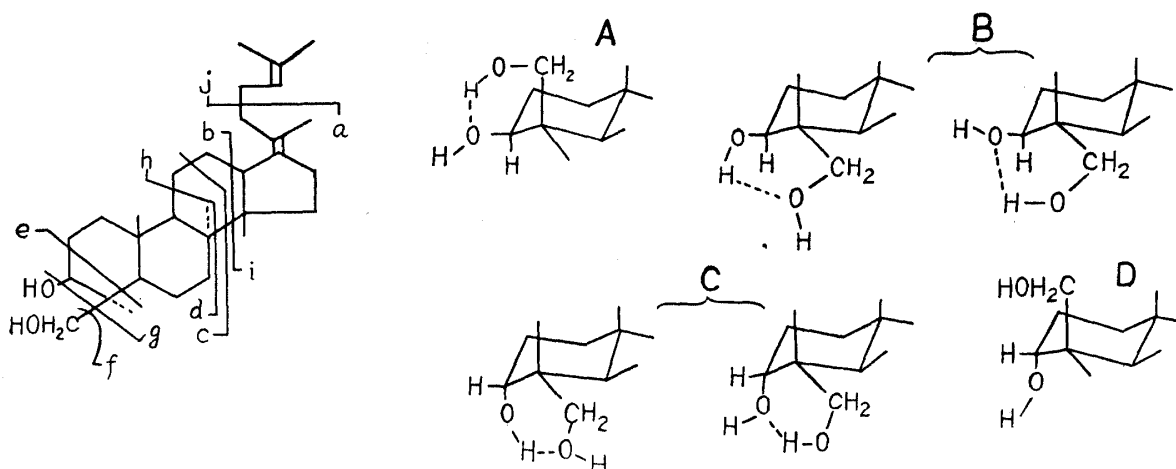
a) The values obtained under addition of D<sub>2</sub>O.

b) These signals were analyzed by the measurement under addition of D<sub>2</sub>O utilizing spin-spin decoupling technique.

structure V can be assigned to it except the stereochemistry of C<sub>3</sub>-OH and C<sub>4</sub>-CH<sub>2</sub>OH, which was elucidated as described below.

This proposed structure can rationalize the mass spectral data of this diol. The high resolution mass spectrum showed the peaks [C<sub>n</sub>H<sub>m</sub>O<sub>2</sub>: 442(M), 373(a), 250(b-2), 237(c-1), 223(d-1), 101(e-1). C<sub>n</sub>H<sub>m</sub>O<sub>1</sub>: 411(f), 355(a-H<sub>2</sub>O). C<sub>n</sub>H<sub>m</sub>: 394(g), 218(h), 189(i-1), 69(j)].

The orientation of 3β-OH and 4β-CH<sub>2</sub>OH could be assigned by the IR- and NMR-spectral studies. The IR-spectrum of the diol in the diluted CCl<sub>4</sub> solution exhibited only the absorption due to a nonbonded secondary OH of equatorial type at 3628 cm<sup>-1</sup> but a nonbonded axial secondary (3637—3639 cm<sup>-1</sup>) or a nonbonded primary OH (3640—3642 cm<sup>-1</sup>) could not be observed. This fact clearly demonstrates that this compound possesses the partial structure (A: 3β-OH, 4β-CH<sub>2</sub>OH, the preferred form of the axial primary OH away from the axial 10-methyl group), since the absorption due to a non-



bonded primary OH should exist in all the other cases, (B:  $3\beta$ -OH,  $4\beta$ -CH<sub>2</sub>OH, the equilibrium mixture of two forms), (C:  $3\alpha$ -OH,  $4\alpha$ -CH<sub>2</sub>OH, the equilibrium mixture of two forms) and (D:  $3\alpha$ -OH,  $4\beta$ -CH<sub>2</sub>OH, no hydrogen bonding)<sup>3)</sup>.

In the NMR spectrum of diacetate the shape of the signal due to C<sub>3</sub>-H (a slightly diffused triplet,  $J = \text{ca } 8$  cps) is in accordance with that of the  $3\alpha$ -H (axial type, t.,  $J = 8$ ,  $W/2 = 17$ ) of the similar compound such as isoescigenin pentacetate epoxide<sup>4)</sup>. On the other hand the average value ( $\frac{H_a + H_b}{2} = 4.42$ ) of the chemical shifts of C<sub>4</sub>-CH<sub>a</sub>H<sub>b</sub>OAc of the diacetate is very similar to that (4.08—4.30), reported in the case of C<sub>4</sub>-CH<sub>2</sub>OAc of axial type but different from that (ca 3.84) of the equatorial type.<sup>5)</sup> Thus the stereochemistry of  $3\beta$ -OH and  $4\beta$ -CH<sub>2</sub>OH in the diol was also proved from the NMR-spectral data.

Consequently this diol, expected to be the first oxidation product of II, is assigned as  $3\beta$ -hydroxy- $4\beta$ -hydroxymethylfusida-17(20)[16,21-cis],24-diene (V).

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3) A.R.H. Cole and G.T.A. Müller, *J. Chem. Soc.*, 1224 (1959).

4) J.B. Thomson, *Tetrahedron Letters*, 2229 (1965).

5) A. Gaudemer, J. Polensky and E. Wenkert, *Bull. Soc. Chim. France*, 407 (1964).