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STEREOCHEMISTRY OF HELVOLIC ACID

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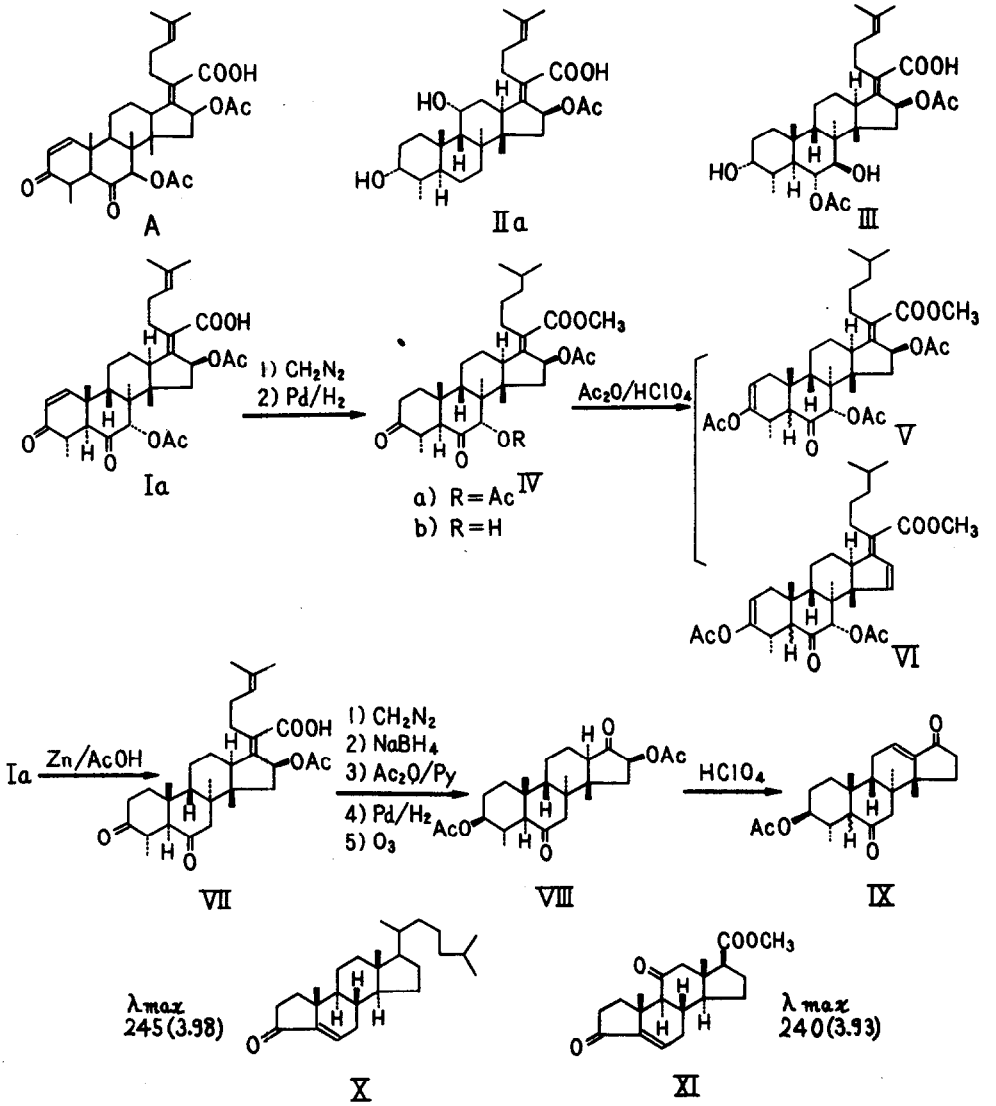
Helvolic acid (Ia) (1) is a fusidane type antibiotic like fusidic acid (IIa) (2) and cephalosporin P_1 (III) (3). Recent publications regarding Ia and III have prompted us to publish the accumulated data*1 on the stereochemistry of this compound.

THE POSITION OF 14-CH_3 Previously we established framework (A) for helvolic acid in which the third tert. CH_3 was tentatively proposed to be located at C_{14} (1b). Its definite proof has been obtained by the enol acetylation of methyl tetrahydrohelvolate (IVa) which afforded two enol acetates (V, VI). V,*2 m.p.207-8°; UV*3 219 (3.85), NMR*4 3 $CO\underline{CH_3}$(7.85,7.90,8.03), C_2-H(4.65 ca., overlaped with C_7-H signal), C_7-H(4.66,s), C_{16}-H(4.11,diffused d,J=8 ca.). VI, m.p.175°; UV 269(4.17), NMR 2 $CO\underline{CH_3}$(7.89,7.94), C_2-H(4.76 ca., overlaped with C_7-H signal), C_7-H(4.70,s), C_{15}- and C_{16}-H(3.06,3.23,d,J=6). NMR spectrum of VI demonstrates that a double bond generated by an elimination of 16-acetoxyl is Δ^{15} and that C_{14} possesses no hydrogen, because the signals of C_{15}- and C_{16}-H are sharp doublets. Consequently the position of third tert. CH_3 is proved to be at C_{14} and the fusidane type framework of Ia thus becomes clear.

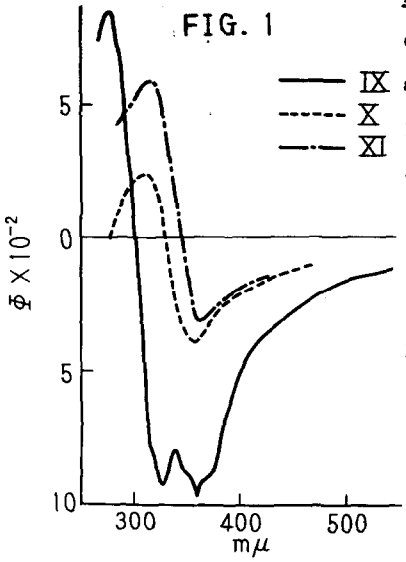
14β-CH_3 Treatment of Ia with zinc dust and acetic acid furnished 7-desacetoxydihydrohelvolic acid (VII) (1b,c) which was converted into Δ^{12}-17-keto derivative (IX) via VIII, m.p.189-92°; NMR 2 $CO\underline{CH_3}$(7.81,7.89) C_3-H(5.52,m), C_{16}-H(4.69,diffused d,J=11

*1 The stereochem. of Ia was partly discussed at 8th symposium on the chemistry of natural products, Nagoya, Japan, 1964(S.Iwasaki, S.Okuda, K.Tsuda, symp. papers p192).
*2 All new compounds cited in this paper gave satisfactory analytical data.
*3 All UV spectra, λmax mμ (logε), were measured in ethanol solution.
*4 τ value and abbreviations (s:singlet, d:doublet, t:triplet, q:quartet, sex:sextet, m:multiplet) are used.

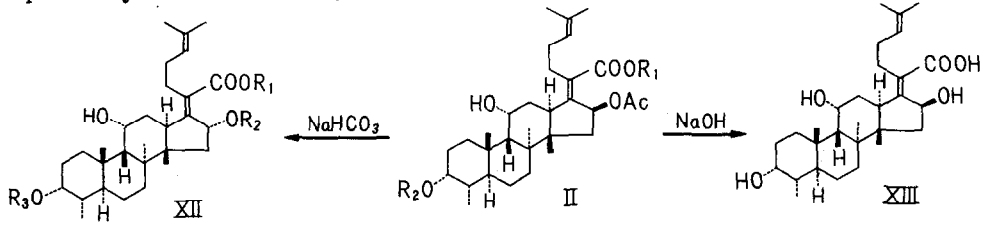


ca.). IX, m.p.188-90°; UV 243(3.98), NMR $COCH_3$(7.98), C_{12}-H(3.45, diffused t, J=3ca.), C_3-H(5.60, m). UV of IX is quite similar to those of 3-keto-5-ene-A-norsteroids such as X and XI (4,5) and the NMR spectrum indicates the existence of only one proton on the double bond. These facts prove this compound to be Δ^{12}-17-ketone. ORD curve of α,β-unsat. ketone of IX shows Cotton effect with the same (-) sign* as those of X and XI, which suggests 14β-CH_3 in IX (FIG.1).

* ORD curve of α,β-unsat. ketone of Δ^{12}-17-ketone from cephalosporin P_1 exhibits also (-) sign (Dr.Halsall's private communication).



16β-OAc The hydrolysis of helvolinic acid (XIVa) on refluxing in 2% $NaHCO_3$-H_2O gave a dihydroxy acid (XVa), m.p.192-3°. Methyl ester (XVb), m.p. 188-8.5°; UV 235(4.20), NMR 3 tert. CH_3(9.22,8.80, 8.49), two C_{25}-CH_3(8.41,8.30),C_4-CH_3(8.79,J=6.5) $COOCH_3$(6.22),C_7-H(6.02,s),C_{16}-H(5.36,diffused t,J= 7.5ca.),C_{24}-H(4.92,m)C_2-,C_1-H(4.18,2.69,both d,J= 10). CD curve*1 due to α,β-unsat. ketone and α-ketol of XVb [θ(dioxane):378(sh,-2260),360(m,-4840) 342(m,-8080),330(m,-11800)] is similar to that of XIVb [θ(dioxane):378(sh,-2120),358(m,-3960),342(sh, -7980),327(m,-9260) . This fact and NMR spectrum of XVb, in which the τ- and J-values of C_1-,C_2-, C_7-H and C_4-CH_3 are almost indentical with those of XIVb (vide infra), demonstrate the same structure of A and B rings in XVb as that of XIVb. Under similar conditions IIa affords 16-epideacetylfusidic acid (XIIa) (6) and by analogy XVa should be 16-epideacetylhelvolinic acid.

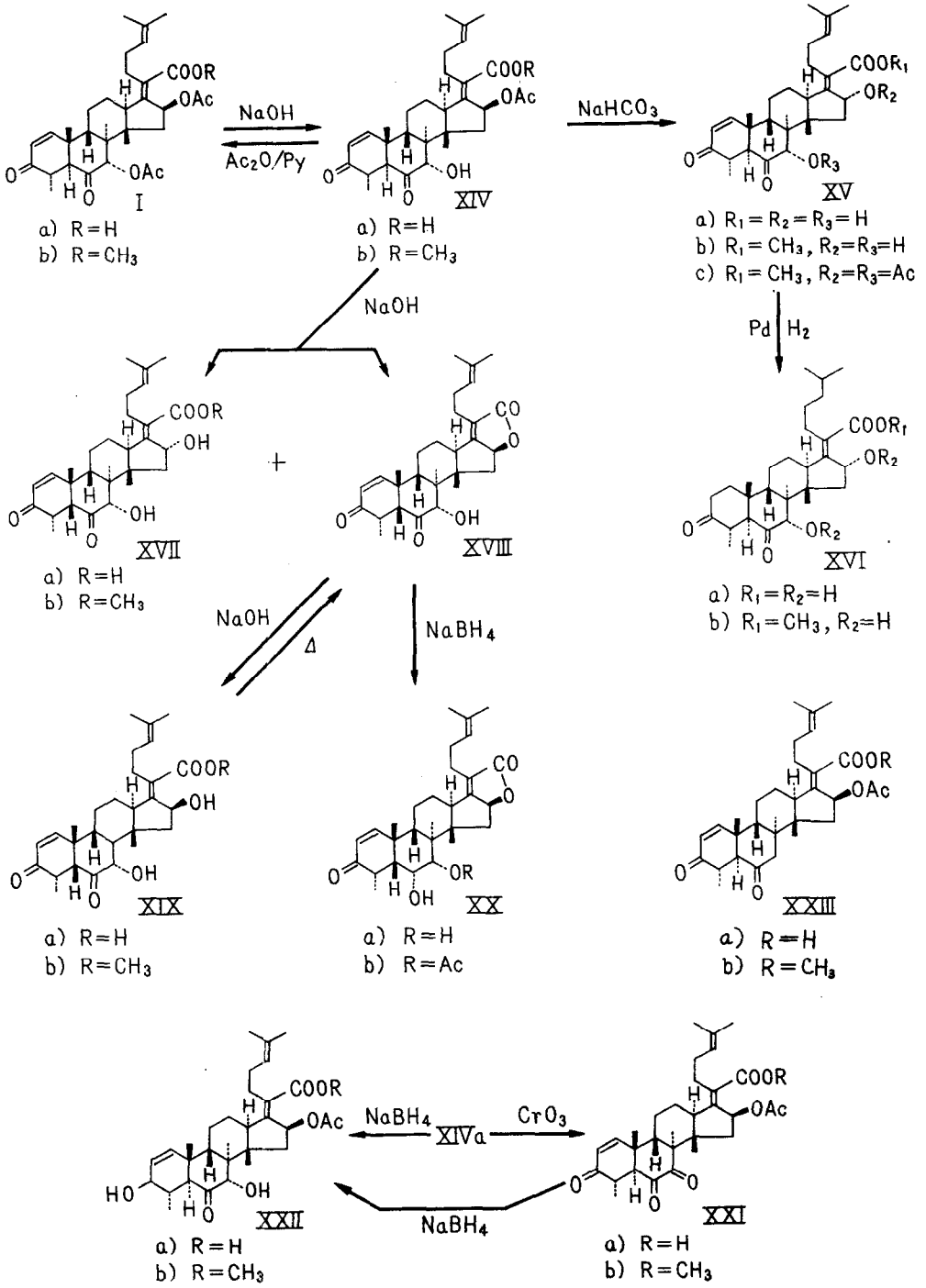


a) $R_1=R_2=R_3=H$
b) $R_1=CH_3$, $R_2=R_3=H$
c) $R_1=H$, $R_2=R_3=Ac$

a) $R_1=R_2=H$
b) $R_1=CH_3$, $R_2=H$
c) $R_1=H$, $R_2=Ac$

1) On comparison between NMR spectra of XIVb, (C_{10}-,C_8-,C_{14}-CH_3:8.44,8.84,9.04),*2 two C_{25}-CH_3(8.38,8.29),C_4-CH_3(8.76,d,J=6.5),$COCH_3$(8.01),$COOCH_3$(6.37),C_7-H(6.0,s),C_{24}-H(4.87,m),C_{16}-H(4.14,overlaped with C_2-H signal),C_2-,C_1-H(4.14,2.65,both d,J=10), and XVb (C_{10}-,C_8-,C_{14}-CH_3:8.49,8.80,9.22),*2 the signal of 14β-CH_3 of XVb shifts to higher field by 0.18 ppm than that of XIVb. This shift is attributed to the conversion of 16β-OAc to 16α-OH and is in good agreement with the higher field shift on compari-

*1 Molecular ellipticity is cited and abbreviations (m:maximum,sh:shoulder) are used.
*2 The assignments of these tert. CH_3 signals have been performed through the comparison of various derivatives of Ia and the details will be published elsewhere.



I a) R=H b) R=CH3

XIV a) R=H b) R=CH3

XV a) R1=R2=R3=H b) R1=CH3, R2=R3=H c) R1=CH3, R2=R3=Ac

XVII a) R=H b) R=CH3

XVIII

XVI a) R1=R2=H b) R1=CH3, R2=H

XIX a) R=H b) R=CH3

XX a) R=H b) R=Ac

XXIII a) R=H b) R=CH3

XIVa

XXII a) R=H b) R=CH3

XXI a) R=H b) R=CH3

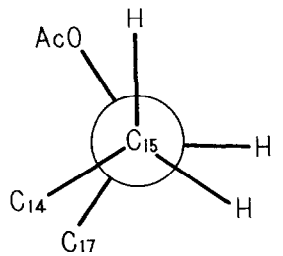
son between IIb (14β-CH_3:9.08 or 9.02) (7) and XIIb (14β-CH_3:9.26) (2).

2) CD curve due to α,β-unsat. carboxylic acid ester of [IVb, θ(CH_3OH):250(+12900), 240(m,+20000),228(0),220(-18800)] and methyl fusidate [IIb, θ(CH_3OH):250(+6400),240 (m,+14000),228(0),220(-21400)] are similar. On the other hand those of methyl 16-epideacetylfusidate [XIIb, θ(CH_3OH):250(-7100),240(-9000),230(-21800),220(-16800)], and methyl 16-epideacetyltetrahydrohelvolinate [XVIb, θ(CH_3OH):250(0),240(-9100), 230(-12600),220(-11000)] also resemble to each other but are different from those of the former pair.

3) ΔM_D in $CHCl_3$ (+216°) between methyl helvolate (Ib) and methyl 16-epihelvolate (XVc) is equivalent to that in $CHCl_3$ (+307° calculated from the data in literature 6) between fusidic acid-3-acetate (IIc) and 16-epifusidic acid-3-acetate (XIIc).

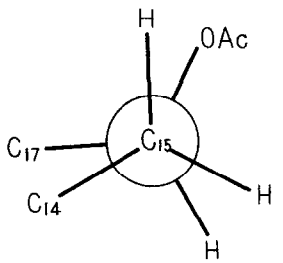
These facts are the rigorous proof for 16β-OAc in Ia.

13α-H The configurations of 14β-CH_3 and 16β-OAc are assigned. Accordingly, as shown in FIG. 2, NMR signal of 16α-H in the configuration with 13β-H should be a quartet while in that with 13α-H it is expected to be a doublet because the dihedral angle between 16α-H and 15β-H is approximately 90° in this case. The signal of 16α-H in methyl tetrahydrohelvolate (IVa) is slightly diffused doublet (4.17,J=8ca.) which proves 13α-H i.e. C/D trans juncture in Ia. NMR signal (5.36,diffused t,J=7.5ca.) of 16β-H in methyl 16-epideacetylhelvolinate (XVb) also supports this conclusion.



Configuration with 13α-H

FIG. 2



Configuration with 13β-H

10β-CH_3 10β-H in Ia may be concluded from the standpoint of biogenesis.

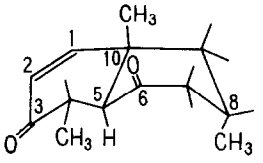
8α-CH_3 AND 5α-H On refluxing in NaOH-H_2O followed by neutralization, XIVa afforded a dihydroxyacid (XVIIa) and a lactone (XVIII) as two main products. Methyl ester XVIIb, m.p.209-11° UV 222(4.27), NMR C_7-H,OH(5.71,6.60,both d,J=5),C_{16}-H(5.35,t,J=7) C_2-,C_1-H(4.24,3.44,both d,J=10). XVIII, m.p.225-7° UV 222(4.23), NMR C_7-H,OH(5.70, 6.70,both d,J=5),C_{16}-H(5.18,q,J=5,11.5),C_2-,C_1-H(4.22,3.43,both d,J=10).

Under similar conditions IIa is converted into 16-deacetyl fusidic acid (XIII)(6) which lactonizes easily in solution. Hydrolysis of XVIII in CH_3OH-NaOH gave a dihydroxy acid (XIXa). Methyl ester (XIXb), m.p.98-9°(monohydrate), UV 222.5(4.23), NMR C_7-H,OH(5.65,6.69,both d,J=5),C_{16}-H(5.36, overlaped with H of H_2O),C_2-,C_1-H(4.25, 3.47,both d,J=10). XIXa regenerates XVIII with great feasibility even during recrystallization. On the other hand, allowing to stand XVIIa in HCl-CH_3OH slowly yielded XVIII. CD curves due to α,β-unsat. ketone of XVIIb [θ(dioxane):344(m,+1500)], XVIII [θ:344(m,+1100)] and XIXb [θ:344(m,+1450)] possess the opposite (+) sign compared to the (-) sign in the case of XIVb and Xvb. Therefore the inversion of A/B juncture takes place under these conditions.

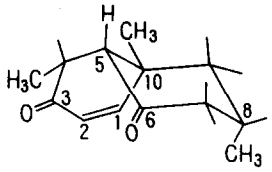
In the case of Ia and XIVa, $NaBH_4$ reduces C_3=O but not C_6=O and catalytic hydrogenation with Pd/C easily furnishes saturation of Δ^1. Contray to this Δ^1 of XVIII cannot be catalytically hydrogenated and $NaBH_4$ reduces C_6=O instead of C_3=O affording XXa. Acetate (XXb), m.p.215-7°, UV 227(4.26), NMR $CO\underline{CH_3}$(7.95),C_6-H(5.88,t,J=4ca.), C_7-H(5.18,d,J=4),C_2-,C_1-H(4.22,3.35,both d,J=10). Therefore Δ^1-3-ketone in XVIII, whose A/B juncture is more stable than that of XIV, is strongly hindered from the attack of these reagents. Regardless with the configuration at C_9, the more stable A/B juncture with 8α-CH_3 is cis and the less stable one is trans, since B-ring in the latter is boat and that in the former chair (FIG.3). In the more stable form with A/B cis juncture, α-side of Δ^1-3-ketone from which reductions take place is completely hindered by B-ring and 8α-CH_3. On the other hand, in the structure with 8β-CH_3 the stable form, whose B-ring is chair, possesses A/B trans juncture, while the less stable one, whose B-ring is boat, has A/B cis juncture (FIG.3). In this case inert-

FIG. 3

Configuration with 8α-CH_3

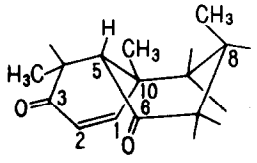


unstable

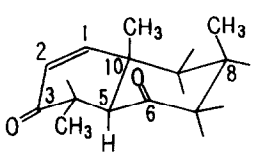


stable

Configuration with 8β-CH_3



unstable



stable

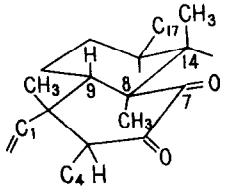
ness of Δ^1-3-ketone of the more stable compound cannot be explained. Consequently the configurations of 8α-CH_3/5β-H in XVIII and 8α-CH_3/5α-H in Ia are concluded.

9β-H Oxidation of XIVa with CrO_3 gave a triketone (XXIa), m.p.204-5°; UV 225(4.22), 330(shoulder,1,82),420(1.52), which on $NaBH_4$-reduction yielded mainly 3-dihydrohelvolinic acid (XXIIa), a sole product obtained also by similar treatment of XIVa. Under these conditions A/B ring juncture should not be changed since 3-dihydrohelvolic acid, the only $NaBH_4$-reduction product of Ia, can reproduce Ia by CrO_3-oxidation and therefore XXIa possesses the original A/B trans juncture.

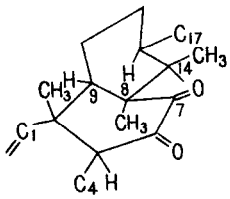
Now, if XXIa has 9α-H, the newly introduced 7-ketone would be located at the position which might cause strong deshielding effect towards 14β-CH_3. Conversely if the configuration of 9-H is β, the τ-value of 14β-CH_3 is not expected to change greatly (FIG.4). In NMR spectra of XIVb (vide supra) and XXIb, m.p.212-3°; 3 tert. CH_3(9.13,8.70,8.60), two C_{25}-CH_3(8.44,8.35),C_4-CH_3(8.87,d,J=7),$COCH_3$(8.10),$COOCH_3$(6.44),C_5-H(7.10, diffused sex,J=7,12),C_6-H(6.53,d,J=12),C_{24}-H(5.00,m),C_{18}-H(4.25,diffused d,J=8.5),C_2-,C_1-H(4.15,2.75,both d,J=10), the signals of 14β-CH_3 appear at 9.04 (XIVb) and 9.13 (XXIb) respectively. This suggests 9β-H in XXIb and then in Ia.

FIG. 4

Configuration with 9β-H



Configuration with 9α-H



4α-CH_3 As already mentioned the stable 4α-configuration of this CH_3 is proposed because this configuration does not change during the alkaline treatment to give XIVa. Futhermore J-value between 4- and 5α-H in XXIb, 12cps, clearly shows axial 4β-H and equatorial 4α-CH_3 in this compound. Consequently 4α-CH_3 in Ia is now elucidated.

7α-OAc Recently a British worker discussed the inertness towards acylation of hindered 6β- and 7β-OH in cephalosporin P_1 derivatives and suggested 7α-OAc in Ia(2b).

Previously we isolated 7-desacetoxy helvolic acid (XXIIIa) (1c), in which τ-value of C_{14}-CH_3 (9.10 in XXIIIb) is similar to that(9.08) of XIVb. If 7-OH in XIVb is β, 14β-CH_3 should be affected by the deshielding effect of 7β-OH and the signal of this CH_3 is expected to appear lower by 0.2-0.3 ppm (δ) than that of 14β-CH_3 in XXIIIb. This is not in accordance with the experimental results and 7α-OH configuration in XIVb is thus assigned.

From the facts mentioned above and the biogenetic standpoint fusidane type framework of helvolic acid is elucidated and the complete stereochemistry of this antibiotic is assigned as Ia.

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REFERENCES

1) a N.L.Allinger and J.L.Coke, J. Org. Chem., 26, 4522 (1961). Literature cited in this paper.
b S.Okuda, S.Iwasaki, K.Tsuda, Y.Sano, T.Hata, S.Udagawa, Y.Nakayama, H.Yamaguchi, Chem. Pharm. Bull. Tokyo , 12, 121 (1964).
c S.Okuda, Y.Nakayama and K.Tsuda, Chem. Pharm. Bull. Tokyo , 14, 436 (1966)

2) W.O.Godtfredsen, W.Von Daehne, S.Vangedal, A.Marquet, D.Arigoni, A.Melera, Tetrahedron, 21, 3505 (1965). Literature cited in this paper.

3) a T.G.Halsall, E.H.Jones, G.Lowe, C.E.Newall, Chem. Comm., 19, 685 (1966). Literature cited in this paper.
b P.Oxley, Chem. Comm., 20, 729 (1966).

4) R.M.Moriarty and E.S.Wallis, J. Org. Chem., 24, 1274 (1959).

5) J.Fried, E.F.Sabo, J. Amer. Chem. Soc., 84, 4356 (1962).

6) W.O.Godtfredsen and S.Vangedal, Tetrahedron, 18, 1029 (1962).

7) A.Melera, Experientia, 19, 565 (1963).

8) Y.Kawazoe, Y.Sato, M.Natsume, H.Hasegawa, T.Okamoto, K.Tsuda, Chem. Pharm. Bull. Tokyo , 10, 338 (1962).