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PIMARICIN—VII

THE ABSOLUTE CONFIGURATION AT C-25[1]

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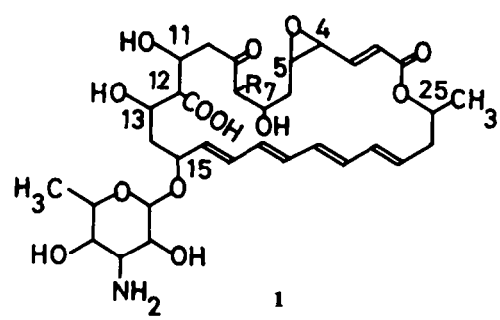
Abstract—The absolute configuration at C-25, the lactone forming carbon atom, in the polyene macrolide antibiotic pimaricin has been established as *R* by isolation of (3*R*)-1,3-diacetoxybutane obtained by ozonolysis followed by reductive work-up and acetylation.

THE polyene macrolide antibiotic pimaricin **1**(R=H)[2-4] contains at least 8 asymmetric carbon atoms in the aglycone part. Of these the configuration at C-25, the secondary carbon atom involved in the formation of the macrocyclic lactone, appears to be the one most easily accessible by methods of chemical degradation. In other polyene macrolides where the corresponding configurations have been determined (lagosin[5], fungichromin[6], rimocidin[7] and lucensomycin[8]), the method of choice has been oxidative cleavage of the terminal double bond of the polyene system, followed either by hydrolysis to give a "small" hydroxy acid or by reductive work-up to yield the corresponding diol. The diols have then been directly compared with authentic samples of known absolute configuration. The second approach has been followed in this investigation.



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[1] Part VI: *Acta Chem. Scand.* **18**, 126 (1964).

[2] J. B. Patrick, R. P. Williams and J. S. Webb, *J. Am. Chem. Soc.* **80**, 6689 (1958).

[3] [a] O. Ceder, J. M. Waisvisz, M. G. van der Hoeven and R. Ryhage, *Chimia* (*Aarau*) **17**, 352 (1963); [b] O. Ceder, *Acta Chem. Scand.* **18**, 126 (1964).

[4] B. T. Golding, R. W. Rickards, W. E. Meyer, J. B. Patrick and M. Barber, *Tetrahedron Letters* 3551 (1966).

[5] M. P. Berry and M. C. Whiting, *J. Chem. Soc.* 862 (1964).

[6] A. C. Cope, R. K. Bly, E. P. Burrows, O. J. Ceder, E. Ciganek, B. T. Gillis, R. F. Porter and H. E. Johnson, *J. Am. Chem. Soc.* **84**, 2170 (1962).

[7] A. C. Cope, E. P. Burrows, M. E. Derieg, S. Moon and W. D. Wirth, *J. Am. Chem. Soc.* **87**, 5452 (1965).

[8] G. Gaudiano, P. Bravo and G. Mauri, *Chim. Ind.*, (*Milan*) **48**, 1327 (1966).

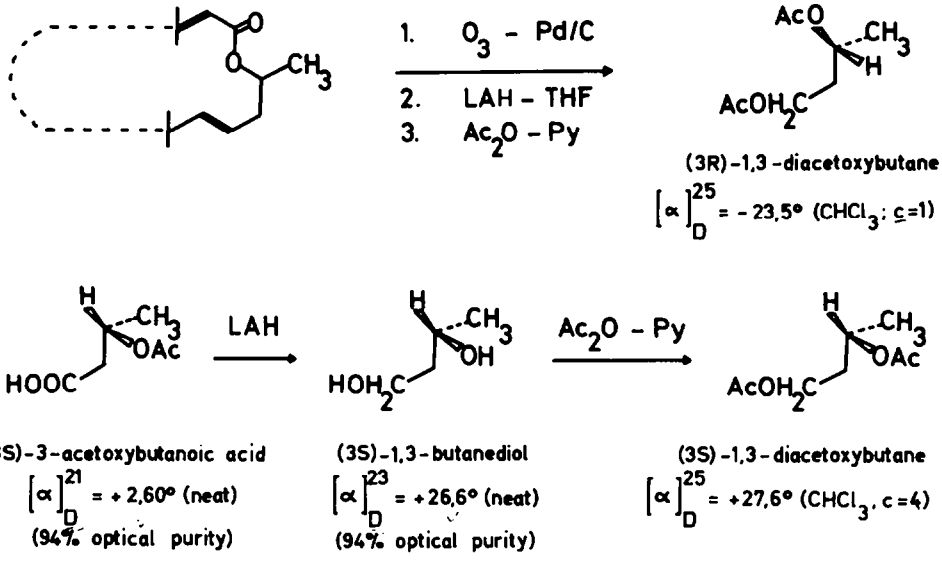
Pimaricin was ozonized at $-50°$ and the ozonide decomposed by catalytic hydrogenation. Reduction of the total reaction mixture with LAH gave a mixture containing 1,3-butanediol. After treatment with acetic anhydride in pyridine, the diol, in form of its diacetate, was isolated by extraction. The crude product was purified by distillation followed by preparative VPC. Analytical VPC showed the presence of only one component with a retention time identical with that of authentic 1,3-diacetoxybutane.[9] The identity of the two samples was further ascertained by TLC, and by mass and IR spectroscopy. The degradative scheme is outlined in Fig. 1.

The 1,3-diacetoxybutane obtained from pimaricin was optically active and levorotatory, $[\alpha]_D^{25} = -23{\cdot}5°$. The authentic sample of 1,3-diacetoxybutane, which was dextrorotatory, $[\alpha]_D^{25} = +27{\cdot}6°$, had been prepared from (3*S*)-1,3-butanediol (cf. Fig. 1). Thus the absolute configuration at C-25 in pimaricin is *R* according to the Cahn-Ingold-Prelog convention.[10]

Levene and Haller[11] (cf. also Refs 12, 13 and 14) have earlier shown that dextrorotatory 1,3-butanediol possesses *S*-configuration by relation to glyceraldehyde *via* 3-hydroxybutanoic and lactic acid.

Recently Gaudiano *et al.*[8] presented data, obtained in a slightly different way, which also lead to the conclusion that pimaricin possesses *R*-configuration at C-25.[15]

Finally, attention might be drawn to the observation that the macrolide antibiotics which have been investigated to date all possess *R*-configuration at the secondary



[9] M. S. Kulpinski and F. F. Nord, *J. Org. Chem.* **8**, 266 (1943).
[10] R. S. Cahn, C. K. Ingold and V. Prelog, *Experientia* **12**, 81 (1956).
[11] P. A. Levene and H. L. Haller, *J. Biol. Chem.* **69**, 165 (1926).
[12] K. Serck-Hanssen, S. Ställberg-Stenhagen and E. Stenhagen, *Arkiv Kemi* **5**, 203 (1952).
[13] K. Serck-Hanssen, *Arkiv Kemi* **8**, 401 (1954).
[14] J. A. Mills and W. Klyne, in *Progr. Stereochem.* (Edited by W. Klyne) Vol. 1, p. 182. Butterworth, London (1954).
[15] We are indebted to Professor G. Gaudiano for sending us a manuscript of their article.

carbon atom forming the lactone.[16] This could indicate that the formation of the lactone bond is not a terminal process, but instead an early step in the biosynthesis of a macrolide. The closure of the macrocyclic lactone would then result from the formation of a carbon–carbon bond (cf. Ref. 17 pp. 12–13 and Ref. 16).

EXPERIMENTAL

IR spectra of the pure liquids (thin film) were determined with a Beckman Model IR9 spectrophotometer. Analytical VPC was performed with a Perkin-Elmer 800 gas chromatograph using a $\frac{1}{8}$ in. × 2 m column packed with 1,4-butanediol succinate on 80–100 mesh HMDS Chromosorb W. Preparative gas chromatographic separations were achieved with an Aerograph Autoprep Model A-700 on a $\frac{3}{8}$ in. × 20 ft. column filled with 30% SE-30 silicon rubber (General Electric Co.) on 45–60 mesh Chromosorb W. N_2 was used as a carrier gas. Mass spectra were determined with an LKB 9000 instrument. TLC was carried out on Silicagel GF (Merck) according to Stahl.[18] The spots were made visible with I_2 (cf. Ref. 18, p. 506.) Optical rotations were measured with a Perkin–Elmer 141 polarimeter using a microcell.

Preparation of (3S)-1,3-*diacetoxybutane.* To 91 mg of (3*S*)-1,3-butanediol, $[\alpha]_D^{23} = +26{\cdot}6°$ (ca. 94% optical purity; cf. Ref. 12, p. 405), in 2 ml pyridine, 400 mg Ac_2O was added at room temp. The mixture was refluxed for 5 hr. after which most of the pyridine was distilled off. The residue, 800 mg colorless oil, gave after isolation by preparative gas chromatography at 175°, 41 mg of (3*S*)-1,3-diacetoxybutane, $[\alpha]_D^{25} = +27{\cdot}6°$ ($CHCl_3$; $c = 4$). By analytical gas chromatography and by TLC (benzene–ether 1:1; $R_f = 0{\cdot}54$) only one component was detected in the collected sample.

Isolation of (3R)-1,3-*diacetoxybutane from pimaricin.* A suspension of 4·0 g pimaricin[19] in 150 ml MeOH was ozonized for 6–7 hr at −50°; the ozonide was then decomposed by catalytic hydrogenation overnight, using 10% Pd on C catalyst. After removal of the catalyst, the soln was evaporated to dryness under reduced press at 15°. The yellowish residue was redissolved in 150 ml anhyd THF. The soln was then filtered and the filtrate added to 200 ml anhyd THF containing 6·0 g LAH. The mixture was stirred under reflux for 18 hr; the excess hydride was then destroyed with glacial AcOH. After evaporation to dryness at 50° and reduced press the gray residue (also containing the inorganic material) was suspended in a mixture of 200 ml pyridine and 300 ml ether; 125 ml Ac_2O was added at room temp, and the mixture was then refluxed for 15 hr. The inorganic material, which remained undissolved, was separated by filtration and continuously extracted with 500 ml ether for 15 hr. The combined filtrate and ether extract were acidified with 20% H_2SO_4 aq to pH = 2 and the organic phase separated. The aqueous layer was extracted with 75 ml ether. The combined organic layers were then washed with 10 ml H_2SO_4, 2 × 10 ml water and 5 × 20 ml 10% Na_2CO_3 aq, and finally dried over $MgSO_4$. The soln was concentrated to 10 ml, transferred to a semimicro distillation apparatus, and fractionated at reduced press. A fraction at 50–60°/2mm was shown by analytical gas chromatography and TLC to contain the desired ester. By preparative gas chromatography at 175°, 12 mg (3*R*)-1,3-diacetoxybutane, $[\alpha]_D^{25} = -23{\cdot}5°$ ($CHCl_3$; $c \doteq 1$), was isolated.

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[16] W. D. Celmer in *Biogenesis of Antibiotic Substances* (Edited by Z. Vanek and Z. Hostalek) pp. 99–130. Publishing House of the Czechoslovak Academy of Sciences, Prague (1965).

[17] O. Ceder, *Svensk Kem. Tidskr.* **76**, 428 (1964).

[18] E. Stahl, *Dünnschicht-Chromatographie.* Springer-Verlag, Berlin (1962).

[19] Supplied by the Koninklijke Nederlandsche Gist-en Spiritusfabriek N.V., Delft, Holland.