Riassunto. La struttura cristallina della *p*-iodio anilide della rifamicina Y è stata determinata con metodi tridimensionali a temperatura ambiente. La cella elementare monoclina, gruppo spaziale $P2_1$, ha i parametri $a = 9{,}49$, $b = 18{,}96$, $c = 15{,}30$ Å, $\beta = 90°48'$, e contiene due molecole.

La costituzione e la configurazione della rifamicina Y, $C_{39}H_{47}NO_{15}$, risultano quindi determinate attraverso lo studio cristallografico della sua *p*-iodio anilide. Anche molte informazioni sulla configurazione della rifamicina Y possono essere tratte dalla configurazione allo stato solido di questo suo derivato.

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[19] We are grateful to Professor P. SENSI of Lepetit SpA Research Laboratories, Milan, for making available supplies of the *p*-iodoanilide of rifamycin Y. We also thank Mr. N. OCELLO for his skillful technical assistance. All the calculations were carried out on the Rome University IBM 7040 computer.

Studies on the Possibility of Epoxide Formation from Disubstituted Hexitols

It was demonstrated by ELSON[1] that in the hematological effects of 1,6-dimethanesulphonyl-D-mannitol (Ia, dimesylmannitol, DMM) a myelotoxicity of the myleran type was combined with some features reminiscent of the lymphotoxic effects of epoxides. On this basis he suggested that in vivo DMM may be converted to epoxide intermediates by splitting off methanesulphonic acid. DAVIS and ROSS[2] investigated the hydrolysis of DMM at pH 7.5, and demonstrated the production of epoxide by addition of thiosulphate to the reaction mixture and acidic titration of the alkalinity liberated in the course of the Bunte-salt formation; the scheme is shown in (Ia–III).

With regard to the chemical analogy between dimesylmannitol and 1,6-dihalogeno-hexitols we were greatly interested in what role may be attributed to an epoxide intermediate in the cytostatic action of dibromomannitol (Ib, DBM, Myelobromol®). We applied to DBM the procedure described by DAVIS and ROSS, and we compared the behaviour of DBM with that of DMM and dibromoerythritol (V, DBE).

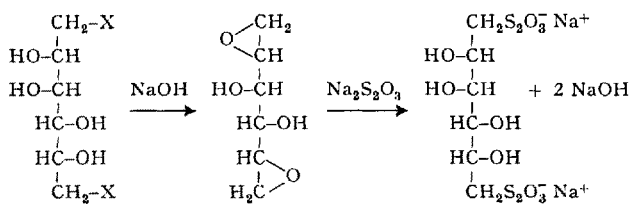
A mixture of 1 m*M* of disubstituted polyol and 10 ml of water was kept at 37 °C and the pH was maintained at 7.5 by continuous addition of 0.1 *N* NaOH as long as the reaction mixture still consumed hydroxide. With DMM and DBM the hydrolysis took 5–6 h, with DBE only $^1/_2$–1 h. To an aliquot part 3–5 g of $Na_2S_2O_3$/m*M* of substance was added, and the alkali set free titrated with 0.1 *N* HCl. The amount of acid required was a measure of the epoxide formed. The calculated epoxide content in the case of DMM, DBM and DBE was 70–75%, 50–55% and 50%, respectively.

These results were inconsistent with the assumed biological role of an epoxide intermediate since the cytostatic activity of DBM is confined selectively to the inhibition of the myeloid system[3,4] in contrast with the lymphotoxic character of the epoxides.

Our attempts to isolate the diepoxide of mannitol, or any other identifiable product, from the reaction mixtures were unsuccessful, and it seemed to us somewhat improbable that 1,6-disubstituted hexitols should yield a product containing energetically unfavourable 3-membered epoxide rings although the presence of 4 hydroxyl groups could give rise to the formation of non-strained 5- or 6-membered internal anhydrides.

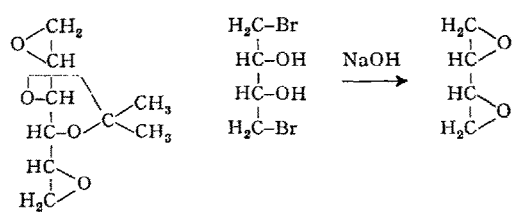
To settle the question, hydrolysis of DMM was carried out in heavy water with NaOD, and NMR measurements were performed on the reaction mixture immediately after the alkaline treatment.

The identification of epoxide rings by proton resonance spectroscopy is based on the observation[5] that epoxide



(Ia) X = OSO_2CH_3 (II) Diepoxide (III) Bunte's salt
(Ib) X = Br

Tentative reaction scheme for the hydrolysis of 1,6-disubstituted hexitols.



(IV) (V) (VI)

1,2-5,6-Dianhydro-3,4-isopropylidene-D-mannitol — 1,4-Dibromo-1,4-dideoxy-meso-erythritol — Meso-1,2-3,4-diepoxybutane

[1] L. A. ELSON, Rep. Br. Emp. Cancer Campn Yorks. Coun. *II*, 11 (1962).
[2] W. DAVIS and W. C. J. ROSS, Biochem. Pharmac. *12*, 915 (1963).
[3] L. INSTITÓRIS, I. P. HORVÁTH and E. CSÁNYI, Int. Symp. Chemother. *III*, 250 (1963).
[4] L. INSTITÓRIS, S. ECKHARDT, I. P. HORVÁTH and C. SELLEI, Arzneimittel-Forsch. *16*, 45 (1966).
[5] R. A. Y. JONES, A. E. KATRITZKY, J. N. MURRELL and N. SHEPPARD, J. chem. Soc. 2576 (1962).

protons usually resonate at slightly higher fields (δ = 2.8–3.5 ppm) than protons adjacent to oxygen (O-C-H) in acylic and larger-ring cyclic ethers or alcoholic OH functions, for which δ = 3.3–3.7 ppm or 3.5–4.5 ppm respectively, are the characteristic ranges.

In the present studies, the diepoxides (IV) and (VI) were used as model compounds regarding the assignation of epoxide proton lines. In the case of (VI) containing only epoxide protons, the spectrum was confined to the 2.4–2.8 ppm range. The observed vicinal coupling constants belonging to the multiplet structure have comparatively small values characteristic for epoxide structures[6]. Measurements on (IV) led to the same conclusions. The chemical shifts of the epoxide protons were in the 2.5–3.2 ppm range, the O-C-H proton shift belonging to the cyclic ether was 3.7 ppm.

Treatment of DBE (V) with alkali at pH 7.5 yields diepoxy-butane (VI) as an isolable product. In the proton resonance spectra of this reaction mixture the major part of the absorption was really found in the epoxide range (2.7–3.3 ppm). An additional multiplet structure was observed at 3.7 ppm which may indicate that either the conversion of starting molecules was not complete or side reactions were also present. (Chemical assay revealed about 50% yield of epoxide.)

In contrast to findings with the epoxide models, in the spectrum of the reaction product of DMM the protons attached to the carbohydrate skeleton resonated in the 3.4–4.2 ppm range, and no evidence was found to support the presence of an epoxide structure, although the thiosulphate test indicated an 'epoxide'-yield of 70%. The chemical shift of the methyl protons in the reaction product differed from the original value measured in DMM which indicates that the parent molecules were completely transformed in the reaction, but no unequivocal conclusions could be drawn concerning the nature of the reaction product.

Experiments with the cytostatic agents dibromomannitol and dibromodulcitol gave similar results to those obtained with DMM.

From these results it appears that the hydrolysis of dimesylmannitol and dibromohexitols does not yield epoxide products. Although the nature of the products of this in vitro reaction is not cleared up, there are no grounds to suppose that alkylation via an intermediary epoxide plays in vivo an important role in the cytostatic action of these compounds[7].

Zusammenfassung. Die NMR-Spektren der Reaktionsprodukte der alkalischen Hydrolyse des Dimesylmannits bestätigten die bisher angenommene Bildung von Epoxiden nicht.

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Chinoin Factory for Pharmaceutical and Chemical Products and Radiofrequency Spectroscopy Laboratory, Central Research Institute for Chemistry, Hungarian Academy of Sciences Budapest (Hungary),
2nd January 1967.

[6] N. S. Bhacca and D. H. Williams, *Application of NMR Spectroscopy in Organic Chemistry* (Holden-Day Inc., San Francisco, London, Amsterdam 1964), p. 99.

[7] The authors are indebted to Miss M. Kajtár for her participation in the NMR measurements.

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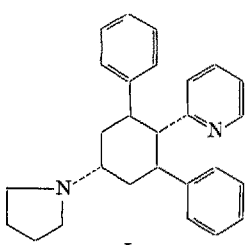
A New Type of Non-Kaliuretic Diuretic Compound

In the course of a structure-study on the alkaloid lobinaline[1] and a synthesis of dehydrolobinaline, a partial dehydrogenation product derived from the alkaloid[2], a considerable excursion into the chemistry of triarylcyclohexanones[3] was required to elucidate the stereochemistry of the degradative and synthetic intermediates involved. Pharmacological screening of these intermediates revealed that 1-(N-pyrrolidino)-3, 5-diphenyl-4-(α-pyridyl)-cyclohexane (I, Su-15049) posseses marked diuretic and natriuretic activity in the rat and dog. The preparation and properties of this compound form the subject of the present report.



I

The initial step in the preparative sequence for Su-15049, which was also directed to a synthesis of the lobinaline ring system[2,3], involved a combination aldol-Michael condensation of 2-phenacylpyridine with benzalacetone to yield 3-hydroxy-3, 5-diphenyl-4-(α-pyridyl)-cyclohexanone. It was subsequently demonstrated[3] by nmr and chemical methods that this substance possesses the all equatorial *trans, trans* conformation of the aryl groups about the central ring. Dehydration of the aldol with phosphoric acid at room temperature yielded *trans*-3, 5-diphenyl-4-(α-pyridyl)-cyclohexen-2-one, which on low pressure hydrogenation with palladium-charcoal catalyst yielded largely *trans, trans*-3, 5-diphenyl-4-(α-pyridyl)-cyclohexanone. For the purpose of attaching a side-chain at position 2 of the ring to effect a synthesis of the lobinaline ring system, the ketone was converted to its pyrrolidine enamine derivative. This enamine was also

[1] M. M. Robison, W. G. Pierson, L. Dorfman, B. F. Lambert and R. A. Lucas, J. org. Chem. *31*, 3206 (1966).

[2] M. M. Robison, B. F. Lambert, L. Dorfman and W. G. Pierson, J. org. Chem. *31*, 3220 (1966).

[3] M. M. Robison, W. G. Pierson, L. Dorfman and B. F. Lambert, J. org. Chem. *31*, 3213 (1966).