

analyses and thermistor molecular weight determinations.

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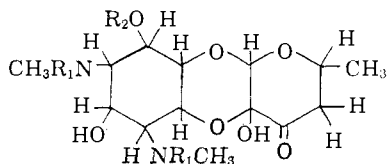
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RECEIVED JULY 11, 1962

CHEMISTRY OF ACTINOSPECTACIN. II. THE STRUCTURE OF ACTINOSPECTACIN

Sir:

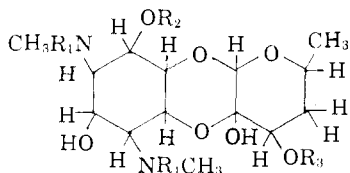
The previous communication¹ in this series described the structure of actinamine, the basic moiety of actinospectacin.²⁻⁵ In this paper Ia is presented as the actinospectacin structure exclusive of stereo-



- Ia, R₁ = R₂ = H
Ib, R₁ = acetyl, R₂ = H
Ic, R₁ = R₂ = acetyl
Id, R₁ = H, R₂ = acetyl
Ie, R₁ = ethylcarbamoyl, R₂ = H

chemistry. Crystalline actinospectacin hexahydrate, m.p. 65–72°, [α]_D 7.6° (c, 1, water), has the molecular formula C₁₄H₂₄N₂O₇·6H₂O,^{6,7} and contains two N–CH₃, one C–CH₃ and two basic groups, pK_a's of 6.95 and 8.70. Besides infrared bands ascribed to hydroxyl and amino hydrogen at 3200–3500 cm.⁻¹, Ia exhibits a carbonyl band at 1735 cm.⁻¹, present only in mulls of rigorously dried samples and absent in crystalline hydrates.

The facile reduction, either with sodium borohydride or catalytic (Pt) hydrogen, of Ia to yield dihydroactinospectacin base, IIa, melting at 83–84°, was further evidence for this carbonyl function.

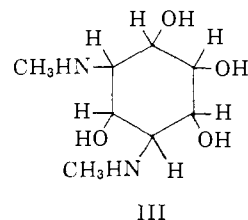


- IIa, R₁ = R₂ = R₃ = H
IIb, R₁ = acetyl, R₂ = R₃ = H
IIc, R₁ = R₂ = R₃ = acetyl

Dihydroactinospectacin contains all the functional groups of its parent antibiotic except the carbonyl function as evidenced by the permanent disappearance of the 1735 cm.⁻¹ band in the infrared. Both Ia and IIa readily form a series of acetyl

derivatives, Ib,c,d and IIb,c, isolated as amorphous solids after countercurrent distribution. The monoacetate, Id, consumes 3 moles of periodate, showing that the acetoxy group cannot be located between the two nitrogen-bearing carbons. Actinospectacin reacts with ethyl isocyanate to produce N,N'-bis-(ethylcarbamoyl)-actinospectacin (Ie) [α]_D -8° (c, 0.84, water), which consumes one mole of periodate.

Methanolic hydrogen chloride fails to cleave actinospectacin or dihydroactinospectacin appreciably. Various acid hydrolyses and mercaptolyses gave actinamine, III, but the remaining six carbons (C₆H₈O₃) were isolated as intractable mixtures



suggesting degradation. Periodate (4 moles) reacts with actinospectacin to give two moles of formic acid and two moles of methylamine. This indication that only three adjacent bonds in the actinamine moiety are available for oxidation was confirmed when N,N'-diacetylactinospectacin, Ib, consumed 1 mole, liberating after hydrolysis actinamine, III, and glyoxylic acid. Two adjacent oxygens, then, are involved in linkages to the remaining fragment(s) totaling six carbons.

From preceding and subsequent data, actinospectacin can be considered to be a glycoside of a relatively unstable, six-carbon, sugar-like substance, designated actinospectose. This moiety as contained in actinospectacin has one C–CH₃, one carbonyl, and at least one non-acylatable hydroxyl.

Dihydroactinospectacin took up four moles of periodate, the diacetyl derivative IIb, one, and the tetraacetyl IIc (in contrast to triacetyl actinospectacin (Ic) which consumes one mole) none. In tetraacetyldihydroactinospectacin the fourth acetate must have been formed at the former carbonyl position. Since this now blocks the periodate reaction, the original carbonyl function must have been adjacent to a hydroxyl. Because the bismuth oxide test for acylloins⁸ is negative for actinospectacin, it must be tertiary.

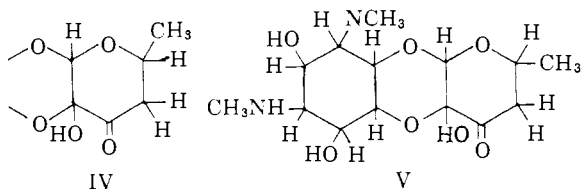
As in the case of the corresponding derivative from actinospectacin, Ib, both actinamine and glyoxylic⁹ acid were isolated from periodate oxidation of IIb. The remaining four carbons were found in the third compound isolated from IIb, crotonaldehyde.⁹ Similarly, crotonic acid was isolated as its *p*-bromophenacyl ester, after periodate oxidation of the actinospectacin derivative, Ie. The new unsaturation must arise from elimination of hydroxyl from a *beta*-carbon. This could not have been a free hydroxyl in actinospectacin as evidenced by a negative iodoform test and failure to acetylate. Partial structure IV for the actinospectose glycoside, linked to actin-

(1) P. F. Wiley, *J. Am. Chem. Soc.*, **84**, 1514 (1962).
(2) A. C. Sinclair and A. F. Winfield, First Interscience Conference on Antimicrobial Agents and Chemotherapy, Oct. 31–Nov. 2, 1961, New York, N. Y.
(3) The trade name of The Upjohn Company for actinospectacin is Trobicin.
(4) D. J. Mason, A. Dietz and R. M. Smith, *Antibiotics and Chemotherapy*, **11**, 118 (1961).
(5) M. E. Bergy, T. E. Eble and R. R. Herr, *ibid.*, **11**, 661 (1961).
(6) Analytical values for all the compounds described in this paper were consistent with the indicated formulas.
(7) Erroneously reported as C₁₄H₂₀₋₂₈N₂O₇ in earlier papers.^{6,7}

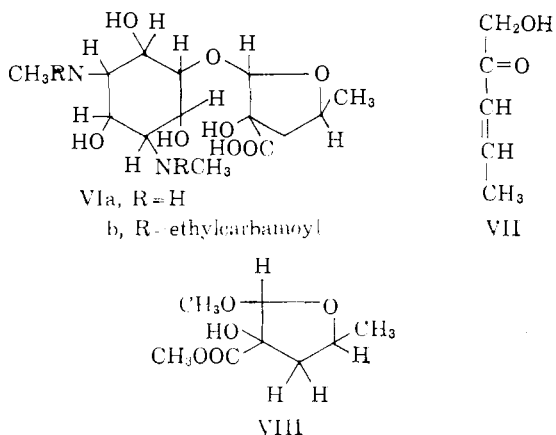
(8) W. Rigby, *J. Chem. Soc.*, 793 (1951).

(9) Isolated as the 2,4-dinitrophenylhydrazone.

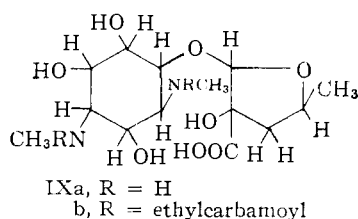
amine either as in I or as in V, explains these products.



Mild base hydrolysis rearranges actinospectacin to actinospectinoic acid, $C_{14}H_{26}N_2O_8$ (VIa), melting $230-235^\circ$, pK_a 3.30 (acidic), and 7.37, 9.33 (basic), and reducing four moles of periodate. Acid cleaves VIa to actinamine, carbon dioxide, and a five-carbon, optically active compound which was isolated as a 1,2-bis-2,4-dinitrophenylhydrazone, $C_{17}H_{16}N_8O_9$, suggesting a 1,2-dicarbonyl or an α -hydroxy carbonyl compound. More vigorous hydrolysis yielded the five-carbon fragment as the unsaturated compound VII, isolated and characterized as its 1,2-bis-2,4-dinitrophenylhydrazone. After borohydride reduction of VII to produce the glycol (not isolated) periodate oxidation gave formaldehyde⁹ and crotonaldehyde.⁹ Methanolysis of VIa afforded actinamine and neutral, optically active VIII ($C_8H_{14}O_5$), which contains two methoxyls, consumes no periodate, and exhibits infrared



absorption in the ester carbonyl region. The formation of VI must be due to a special example of the tertiary ketol rearrangement¹⁰ involving cleavage of the actinamine-actinospectose bond adjacent to the carbonyl. The isolation and behavior of VIII confirm the presence of a six-carbon moiety in actinospectacin. The alternate structure V would rearrange to IXa.



(10) For a review on this subject see S. Selman and J. F. Eastham, *Quarterly Reviews*, **14**, 221 (1960).

The choice between pairs I and V, and VI and IX remains to be made. Base converts N,N' -bis-(ethylcarbamoyl)-actinospectacin, Ie, to the corresponding actinospectinoic acid derivative, VIb, $\alpha_D -63^\circ$ (c , 1, water). Since VIb consumes no periodate, the alternate structure IXb is excluded. Consequently the choice of VIa is required for actinospectinoic acid, eliminating V, and establishing I as the structure of actinospectacin.

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E.P.R. OF DIPHENYLMETHYLENE, A GROUND-STATE TRIPLET

Sir:

Electron resonance (e.p.r.) due to a triplet state of an organic molecule was first observed by Hutchison and Mangum.¹ The triplet state of naphthalene was detected in this elegant method by measuring the spin resonance of ultraviolet-irradiated single crystals of solid solutions of this compound in durene. These resonance absorptions were attributed to transitions between neighboring sublevels of the triplet ($|\Delta m| = 1$ transitions). Van der Waals and de Groot² observed the e.p.r. due to the $|\Delta m| = 2$ transition of the triplet state of a number of polynuclear aromatic hydrocarbons in irradiated rigid glass solutions at $77^\circ K$.

Recently, Yager, Wasserman and Cramer³ have observed the $|\Delta m| = 1$ transition in ultraviolet-irradiated rigid glass solutions of several aromatic hydrocarbons. The detection of these lines in randomly oriented molecules is possible when the magnetic field is approximately parallel to a molecular axis. This is a simple and fruitful method for the examination of triplet states.

All of the above experiments have been concerned with resonance absorption due to an excited state of a molecule. We now wish to report the detection of e.p.r. absorptions due to both $|\Delta m| = 1$ transitions and $\Delta m = 2$ transitions in a chemical species, diphenylmethylene, which is presumably in its ground state. This constitutes the first physical evidence for the triplet nature of diphenylmethylene, a species of considerable interest in the chemistry of divalent carbon intermediates.

In a typical experiment⁴ a dilute solution (*ca.* $10^{-3} M$) of diphenyldiazomethane in polychlorotrifluoroethylene⁵ was irradiated with a Hanovia 140-w. mercury arc at $77^\circ K$. in the quartz Dewar insert of a Varian 100-kc. modulation e.p.r. spectrometer

(1) C. A. Hutchison, Jr. and B. W. Mangum, *J. Chem. Phys.*, **29**, 952 (1958); **34**, 908 (1961).

(2) J. H. van der Waals and M. S. de Groot, *Mol. Phys.*, **2**, 233 (1959); **3**, 191 (1960).

(3) W. A. Yager, E. Wasserman and R. M. R. Cramer, *J. Chem. Phys.*, in press.

(4) The authors wish to acknowledge the assistance of Mr. Richard M. R. Cramer in these experiments.

(5) Obtained from the Hooker Chemical Company as "Fluorolube." No resonance absorption was observed when this material was irradiated alone. Nujol glasses gave similar resonances.