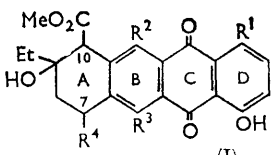
747. Θ-*Rhodomycinone.*

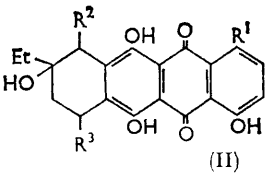
By J. H. Bowie and A. W. Johnson.

An antibiotic preparation from an un-named species of *Streptomyces* has been shown to be a mixture of glycosides and aglycones of the rhodomycin group. One of the aglycones, θ-rhodomycinone, probably contains an equatorial ester group in ring A and is a stereoisomer of the known ε-rhodomycinone.

Since the discovery of the rhodomycin and pyrromycin group of antibiotics by Bockmann and his co-workers,[1] several related antibiotic substances have been recognised including the cinerubins,[2] rutilantin,[3] and aklavin.[4] The aglycones of the antibiotics were studied in detail and they have been shown to be related anthraquinones of the general structure (I), where the substituents R^1, R^2, R^3, and R^4 may be hydrogen or hydroxyl groups. Some members of the group lack the ester substituent at position 10. All the antibiotics of the rhodomycin group yield both amino-sugars and neutral sugars on mild acid hydrolysis.[5]



(I)



(II)

Through the courtesy of Dr. M. Lumb and his colleagues of Boots Pure Drug Co., we have examined a coloured antibiotic, B 5794, which has proved to be a complex mixture

[1] Brockmann *et al.*, *Chem. Ber.*, 1951, **84**, 700; 1953, **86**, 261; 1955, **88**, 1455; 1959, **92**, 1904.
[2] Ettlinger, Gaümann, Hütter, Keller-Schlierlein, Kradolfer, Niepp, Prelog, Reusser, and Zähner, *Chem. Ber.*, 1959, **62**, 1867.
[3] Ollis, Sutherland, and Gordon, *Tetrahedron Letters*, 1959, No. 16, 17.
[4] Gordon, Jackman, Ollis, and Sutherland, *Tetrahedron Letters*, 1960, No. 8, 28.
[5] Brockmann *et al.*, *Naturwiss.*, 1955, **42**, 154; 1961, **48**, 717; 1963, **50**, 43.

of substances of the rhodomycin group containing both glycosides and aglycones. With the aid of reference samples and chromatograms kindly provided by Professor H. Brockmann, the aglycone fraction has been shown to consist mainly of ε-rhodomycinone (I; $R^1 = H$, $R^2 = R^3 = R^4 = OH$),[6,7] ε-isorhodomycinone (I; $R^1 = R^2 = R^3 = R^4 = OH$),[8] and a new rhodomycinone, now named θ-rhodomycinone. Small amounts of ξ- (I; $R^1 = R^4 = H$, $R^2 = R^3 = OH$),[7] ξ-iso- (I; $R^1 = R^2 = R^3 = OH$, $R^4 = H$),[7] and β-rhodomycinone (II; $R^1 = H$, $R^2 = R^3 = OH$) [9] were also detected by chromatography. As we do not propose to carry out further work on this topic we are reporting our experiments on the structure of θ-rhodomycinone at this stage.

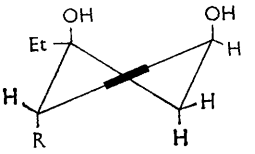
θ-Rhomindoycone, $C_{22}H_{20}O_9$, showed chromatographic behaviour similar to that of δ-rhodomycinone although the two substances were not identical.[10,11]

It was less soluble than either ε- or ε-iso-rhodomycinone in most common organic solvents and its colour reactions and light absorption, both in ethanol and concentrated sulphuric acid, indicated the presence of the 1,4,5-trihydroxyanthraquinone of the rhodomycinone rather than the pyrromycinone type.[6] It contained one methoxyl group which was present as a methoxycarbonyl group showing infrared carbonyl absorption in chloroform at 1712 cm.$^{-1}$. Most rhodomycinone ester carbonyl groups absorb in the infrared at about 1725—1740 cm.$^{-1}$ and as the visible absorption spectrum of θ-rhodomycinone shows a hypsochromic shift of about 4 mμ in the 480—535 mμ region compared with 1,4,5-trihydroxyanthraquinone, it seemed that the ester group was probably hydrogen bonded with an adjacent phenolic hydroxyl group. Kuhn–Roth oxidation gave both acetic and propionic acids, indicative of the presence of an ethyl side-chain. The action of cold pyridine and acetic anhydride on θ-rhodomycinone gave a tetra-acetate, the infrared absorption of which showed a band at 3610 cm.$^{-1}$ suggestive of a non-acetylatable, probably tertiary, hydroxyl group. Bands in the carbonyl region were assigned to phenolic acetate (1775), alcoholic acetate (1741), unbonded methyl ester (1732), and quinone (1672 cm.$^{-1}$). The nuclear magnetic resonance spectrum of θ-rhodomycinone tetra-acetate in chloroform showed bands at τ 8·85(3 protons), 8·40(2), 7·84(3), 7·56(11), 6·36(3), 5·80(1), and 4·55(1). The first two of these showed the characteristic pattern of an isolated ethyl group; a sharp band at τ 7·84 was associated with the alcoholic acetate methyl group and the band at τ 7·56 with three phenolic acetate methyl groups. This band was superimposed on a broad band which was attributed to a methylene group not attached to an aromatic ring. The remaining three bands were associated with the ester methyl group (τ 6·36) and protons on the carbon atoms bearing the ester (τ 5·80) and the alcoholic acetate groups (τ 4·55).[4] The foregoing evidence suggested that θ-rhodomycinone was stereoisomeric with ε-rhodomycinone (I; $R^1 = H$, $R^2 = R^3 = R^4 = OH$), and on biogenetic grounds, probably differs only in the configuration of the ring A substituents.

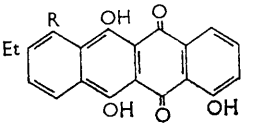
On the basis of the results of circular dichroism measurements and known chemical reactions, Brockmann and Legrand [12] have assigned the absolute configuration (III; R = OH or CO_2Me) to ring A of the pyrromycinones and rhodomycinones, the heavy line signifying the connection to the aromatic ring. When ε-, ε-iso-rhodomycinone or ε-pyrromycinone (I; $R^1 = R^3 = R^4 = OH$, $R^2 = H$) [2,3,13,14] was heated with hydrogen bromide in glacial acetic acid, elimination of two mols. of water occurred and the corresponding tetracenequinones were formed.[2,3,7,8] No hydrolysis of the axial ester groups occurred because of the steric effects of bulky adjacent substitutents. However, if the ester had

[6] Brockmann and Franck, *Chem. Ber.*, 1955, **88**, 1792.
[7] Brockmann and Brockmann, *Chem. Ber.*, 1961, **94**, 2681.
[8] Brockmann and Boldt, *Chem. Ber.*, 1961, **94**, 2174.
[9] Brockmann, *et al.*, *Naturwiss.*, 1957, **44**, 616; *Chem. Ber.*, 1963, **96**, 1356.
[10] Professor H. Brockmann, private communication.
[11] Brockmann and Brockmann, *Chem. Ber.*, 1963, **96**, 1771.
[12] Brockmann and Legrand, *Tetrahedron*, 1963, **19**, 395.
[13] Brockmann and Lenk, *Chem. Ber.*, 1959, **92**, 1880.
[14] Brockmann, Brockmann, Gordon, Keller-Schlierlein, Lenk, Ollis, Prelog, and Sutherland, *Tetrahedron Letters*, 1960, No. 8, 25.

been in the equatorial position, hydrolysis should have occurred readily and this was found to be the case with θ-rhodomycinone. With hydrogen bromide in glacial acetic acid several products were formed, none of which contained ester groups. The tetracenequinone (IV; R = H), bisanhydro-γ-rhodomycinone, was the most significant product, although a carboxylic acid was also isolated which gave (IV; R = H) on heating. The quinone (IV; R = H) has also been obtained from both β- and γ- (II; $R^1 = R^3 = H$; $R^2 = OH$) rhodomycinones.[15] Treatment of θ-rhodomycinone with N-sodium hydroxide under reflux gave the same products and attempts to obtain bisanhydro-ε-rhodomycinone (IV; R = CO_2Me) failed.



(III)



(IV)

On this basis, the structure (I; $R^1 = H$, $R^2 = R^3 = R^4 = OH$) is suggested for θ-rhodomycinone, with the ester group in the equatorial position, a feature which is unique in the rhodomycinones so far described. The configurations of the other substituents of ring A have not been determined.

Although a preliminary study has been made of the purification and nature of the glycosidic fraction, no novel features have emerged. The amino-sugar, rhodosamine, has been identified in the hydrolysis products by chromatographic comparison with an authentic specimen [5] and the aglycone proved to be a mixture of γ- and γ-iso-rhodomycinones [9] which, with hydrogen bromide in acetic acid gave bisanhydro-γ-rhodomycinone (IV; R = H), identical with the product obtained similarly from θ-rhodomycinone.

Experimental

Melting points were measured on a Kofler hot-stage apparatus and are not corrected. Infrared spectra were measured in potassium bromide discs on a Unicam S.P. 100 spectrophotometer and ultraviolet spectra, in 95% ethanol (unless otherwise specified), were recorded with a Perkin-Elmer 137UV instrument. Nuclear magnetic resonance spectra were measured with an AEI/RS2 instrument operating at 60 Mc./sec., with tetramethylsilane as an internal reference, and were calibrated by the side band technique. Individual rhodomycinones were identified by circular paper chromatography on Whatman seed paper using the solvent system, decalin–tetralin–acetic acid–water (5 : 5 : 10 : 1, top layer).[10] Light petroleum refers to the fraction of b. p. 60—80°.

Extraction of Antibiotic B 5794.—The antibiotic (210 g.) was exhaustively extracted with chloroform (Soxhlet). The material (155 g.) remaining after the extraction was only weakly active against *Staphylococcus aureus*. The semi-solid material (54 g.), obtained by concentration of the chloroform extract, was exhaustively extracted (Soxhlet) with ether. Concentration of the ethereal extract gave semi-solid material (19·2 g.), which was a mixture of waxes and aglycones, while the purple solid remaining (34·8 g.) consisted of a mixture of hydroxyquinone glycosides.

The aglycone fraction (19·2 g.) was chromatographed on a column of silica gel (MFC; Hopkin and Williams Ltd.) (60 × 3 cm.). Elution with benzene removed fats and waxes (15·6 g.); elution with benzene–chloroform (7 : 3) gave a mixture of ε- and ε-iso-rhodomycinone (1·25 g.), and, finally, elution with chloroform gave θ-rhodomycinone (145 mg.).

ε-Rhodomycinone (I; $R^1 = H$, $R^2 = R^3 = R^4 = OH$).—The mixture of ε- and ε-isorhodomycinone (1·25 g.) was fractionally crystallised several times from benzene, and the crystalline product was chromatographed on silica gel (60 × 2 cm.). The main band was eluted with benzene–chloroform (9 : 1), and concentration of this fraction gave ε-rhodomycinone (31 mg.) which crystallised from benzene as rosettes of deep red needles, which decomposed at 216—218° (lit.,[5,6] 210°) (Found: C, 61·7; H, 4·65. Calc. for $C_{22}H_{20}O_9$: C, 61·65; H, 4·7%). The infrared and ultraviolet spectra were identical with those reported by Brockmann.[6,7] The tetra-acetate

[15] Brockmann and Niemeyer, *Naturwiss.*, 1961, **48**, 570.

crystallised from methanol as pale yellow needles, m. p. 217—219° (lit.,[6,7] 219°). (Found: C, 60·8; H, 4·65. Calc. for $C_{30}H_{28}O_{13}$: C, 60·4; H, 4·75%).

η-Isopyrromycinone.—The mother-liquors from the ε-rhodomycinone preparation were similarly chromatographed on silica gel in benzene–chloroform (4 : 1). Concentration of the major fraction gave ε-isorhodomycinone (463 mg.), which separated as an amorphous red powder from benzene, but could not be crystallised and decomposed at 223—226° (lit.,[6,8] 229°). Correct analytical figures were not obtained, but ε-isorhodomycinone was identified by its R_F value,[10] infrared spectrum,[6] and ultraviolet spectrum.[6] Treatment of crude ε-isorhodomycinone (100 mg.) with hydrogen bromide–acetic acid,[6,13] and chromatography of the products over silica gel (40 × 3 cm.) in benzene, followed by sublimation at 220°/10^{-2} mm., gave η-isopyrromycinone which crystallised from benzene as deep red needles, m. p. 229—230° (lit.,[8] 230°) (Found: C, 64·7; H, 4·0. Calc. for $C_{22}H_{16}O_8$: C, 64·7; H, 3·95%); $\lambda_{max.}$ 271, 498, 526, 534, 552, and 564 mμ (ε 36,300, 22,400, 24,000, 23,400, 21,900, and 24,000, respectively), with inflections at 465 and 514 mμ (ε, 17,400 and 20,000, respectively). The infrared spectrum of this compound was identical with that of authentic η-isopyrromycinone, kindly provided by Professor H. Brockmann.

Θ-Rhodomycinone (I; R^1 = H, R^2 = R^3 = R^4 = OH).—The crude Θ-rhodomycinone (see above) was further purified by chromatography over a magnesium carbonate (heavy GPR, Hopkin and Williams) diatomite column (3 : 1, 60 × 5 cm.) in acetone, and was crystallised from chloroform and finally from tetrahydrofuran giving red-brown needles, decomposing at 220°, $[\alpha]_D^{25}$ +191·5 (*c* 0·223 in tetrahydrofuran) (Found: C, 61·3; H, 4·85; OMe, 8·5. $C_{22}H_{20}O_9$ requires C, 61·65; H, 4·7; 1 OMe, 7·3%); $\lambda_{max.}$ 234, 258, 296, 480, 493, 513, and 527 mμ (ε 39,800, 22,400, 6610, 12,600, 13,500, 10,200, and 8910, respectively) with an inflection at 465 mμ (ε 10,200). In concentrated sulphuric acid, Θ-rhodomycinone showed maxima at 528 and 595 mμ. It had $\nu_{max.}$ 3478, 2963, 2878, 2850, 1712, 1642, 1622, 1581, 1515, 1487, 1444, 1389, 1360, 1321, 1281, 1263, 1218, 1180, 1127, 1093, 1057, 1044, 1010, 983, 958, 945, 918, 912, 901, 887, 837, 785, 740, and 712 cm.$^{-1}$. Kuhn–Roth oxidation [16] gave a mixture of acetic and propionic acids.

Θ-Rhodomycinone (20 mg.) was acetylated at room temperature with acetic anhydride and pyridine, and the *tetra-acetate* (22 mg.) crystallised from ethanol as yellow needles, m. p. 224—226° (Found: C, 60·2; H, 4·9. $C_{30}H_{28}O_{13}$ requires C, 60·4; H, 4·75%); $\lambda_{max.}$ 259·5, and 349·5 mμ (ε 22,900, and 5370). The infrared spectrum was similar to, but not identical with that of, ε-rhodomycinone tetra-acetate.

Hydrolysis of Θ-Rhodomycinone.—Θ-Rhodomycinone (20 mg.) was heated under reflux with hydrogen bromide in glacial acetic acid (50%, 5 ml.) for 3 hr. The reaction mixture was poured into water (30 ml.), and the solid obtained by filtration was dried and extracted with benzene (20 ml.). The benzene extract was chromatographed on silica gel (20 × 3 cm.). Elution with benzene gave bisanhydro-γ-rhodomycinone (IV; R = H) (3 mg.), which crystallised from benzene–light petroleum (1 : 1) as red-brown needles, m. p. 206—208° (lit.,[9] 208°). The product was identical (mixed m. p.; visible and infrared spectra) with that obtained similarly (see below) from γ-rhodomycinone.

The benzene-insoluble portion (9 mg.) separated from tetrahydrofuran as an amorphous brown powder; $\nu_{max.}$ at 3560, 2962, 2920, 2625, 1780 (γ-lactone?), and 1706 cm.$^{-1}$. Extraction with aqueous sodium hydrogen carbonate removed the band at 1780 cm.$^{-1}$. Sublimation of the product at 250°/10^{-2} mm. gave bisanhydro-γ-rhodomycinone (IV; R = H).

The Glycosidic Fraction.—The crude glycosides (1·5 g.) were separated by counter-current distribution (100 transfers) using the system: carbon tetrachloride–methanol–water (60 : 51 : 8)[2]. Fractions 5—14, 45—80, and 89—97 were distilled *in vacuo* and gave glycosides *A* (60 mg.), B (302 mg.), and C (805 mg.), respectively. Glycosides A and B separated from benzene as amorphous powders, m. p. 168—170 and 173—175°, respectively, whereas glycoside C, present as the sodium salt, was precipitated from benzene as a violet powder, m. p. >350° (decomp.).

Hydrolysis of the Glycosides.—Each glycoside (10 mg.) was heated with 0·5N-sulphuric acid (5 ml.) on the water-bath for 30 min. Nitrogen was passed through the reaction mixture and the effluent gases were bubbled into barium hydroxide solution. Barium carbonate was immediately precipitated and the reaction mixture gave a positive Nessler test. Filtration of the reaction mixture gave a solid which was shown by chromatography to be a mixture of and γ-iso-rhodomycinone and which could not be separated. The filtrates were neutralised

[16] Reid and Lederer, *Biochem. J.*, 1952, **50**, 60.

with aqueous barium hydroxide and centrifuged, and the supernatant liquid was distilled *in vacuo*. Ascending paper chromatography on a Whatman No. 1 paper in the system, n-butanol–acetic acid–water (4 : 1 : 1) gave the following results: Glycoside A, R_F 0·35, 0·53, 0·76; B, 0·21, 0·53, 0·76; C, 0·21, 0·35, 0·53, 0·76. Extracts of all these spots were positive to ninhydrin, aniline hydrogen phthalate, alkaline silver nitrate, and triphenyltetrazolium chloride spray reagents, with the exception of the spot at R_F 0·21, an extract of which did not react with ninhydrin, and was identified as glucose. The compound with R_F 0·53 was identified as rhodosamine,[5] by chromatographic comparison with an authentic sample, kindly supplied by Professor H. Brockmann.

Bisanhydro-γ-rhodomycinone (IV; R = H).—The mixture of γ- and γ-iso-rhodomycinone (100 mg.) was treated with hydrogen bromide–acetic acid as described above. Chromatography of the products on silica gel in benzene, followed by fractional sublimation at 250°/10^{-2} mm., gave bisanhydro-γ-rhodomycinone, which crystallised from benzene–light petroleum (1 : 1) as red-brown needles (43 mg.), m. p. 207—208°, identical with the specimens already prepared (above) (Found: C, 71·7; 71·5; H, 3·85, 4·35. Calc. for $C_{20}H_{14}O_5$: C, 71·85; H, 4·2%), $\lambda_{max.}$ at 496 and 532 mμ (ε 17,800 and 20,400) with inflections at 466 and 522 mμ (ε 8910 and 17,400). The infrared spectrum was identical with that of authentic bisanhydro-γ-rhodomycinone, provided by Professor H. Brockmann.

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