Notes

Synthesis of ϵ-Rhodomycinone Glycosides

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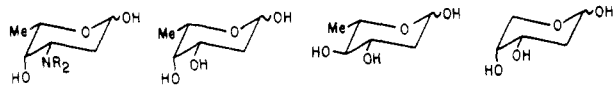
Twenty-six ϵ-rhodomycinone glycosides have been synthesized. These include the ϵ-rhodomycinone glycosides of 2-deoxy-L-fucose, 2-deoxy-L-rhamnose, and 2-deoxy-D-ribose as well as their 2-hydroxyl derivatives. NMR spectroscopy showed that all the glycosides prepared had the saccharide residues linked to position 10 of ϵ-rhodomycinone and helped establish the anomeric purity and configuration of several glycosides. Preliminary screening results show that 2-deoxy-di-*O*-acetyl-D-ribopyranosyl-ϵ-rhodomycinone has an activity T/C of 125 on P388 tumors.

The rhodomycin complex was isolated by Brockmann and Bauer[1] from *Streptomyces purpurascens* who later separated it into (a) glycosides which showed antibiotic properties and (b) inactive aglycons. Mild acid hydrolysis of the glycosides yielded an amino sugar, rhodosamine (1, R = Me),[2] and two deoxy sugars, 2-deoxy-L-fucose (2) and rhodinose (2,3,6-trideoxy-L-*threo*-hexopyranose),[3] as well as two aglycons, β-rhodomycinone and γ-rhodomycinone. The inactive aglycons[4-6] isolated from the fermentation mixture included the α-, δ-, and ϵ-rhodomycinones.

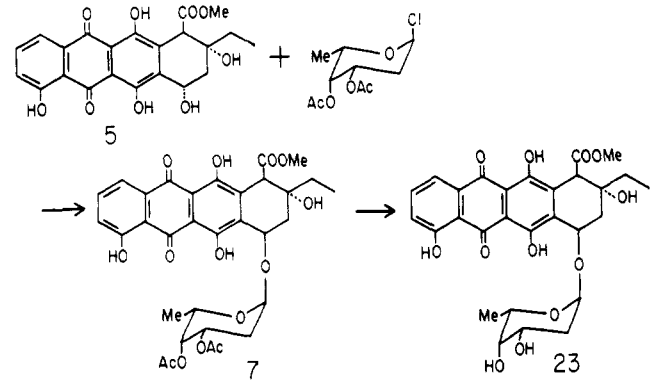
The work described here aimed at studying the possibility of converting ϵ-rhodomycinone (5), an inactive by-product isolated during the manufacture of anthracycline antibiotics by fermentation, into an active glycoside. Henry[7] has recently prepared a moderately active glycoside by linking the amino sugar, daunosamine (1, R = H), to ϵ-rhodomycinone, and it was reasoned that since the active rhodomycins contain both amino sugars and deoxy sugars, one could possibly induce activity by forming the deoxy sugar glycosides of ϵ-rhodomycinone. Three principal deoxy sugars were selected for glycosidation with ϵ-rhodomycinone; they were 2-deoxy-L-fucose (2), which is present in the rhodomycin antibiotics and which may be considered the oxygen analogue of both daunosamine (1, R = H) and rhodosamine (1, R = Me), and 2-deoxy-L-rhamnose (3), which is the 4-epimer of 2-deoxy-L-fucose.



1 2 3 4

The rationale for selecting this epimer was the fact that in the daunorubicin series, the daunomycinone glycosides of the 4-epimer of daunosamine, namely, 3-amino-2,3,6-trideoxy-L-*arabino*-hexopyranose, exhibited marked antitumor activity. The third principal deoxy sugar selected for glycosidation with ϵ-rhodomycinone was 2-deoxy-D-ribopyranose (4). This is because of the structural similarity that exists between 2-deoxy-L-fucose (2) and 2-deoxy-D-ribose (4), with the latter lacking the methyl group attached to C-5 of the former. The 2-deoxy-D-ribofuranoside of ϵ-rhodomycinone was also prepared for screening since no furanosyl derivatives of anthracyclines had been tested. Also included in this study were the 2-hydroxyl derivatives of the three principal target glycosides, namely, the L-fucopyranosyl- and the L-rhamnopyranosyl-ϵ-rhodomycinones, as well as the pentosyl-ϵ-rhodomycinones in the pyranose and furanose forms.

Synthesis of the ϵ-Rhodomycinone Glycosides 6–31. At the time this work was initiated, a paper by Henry[8] appeared describing the formation of daunorubicin by reacting the appropriate glycosyl halide with daunomicinone in the presence of mercuric salts. ϵ-Rhodomycinone



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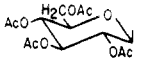
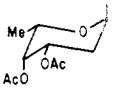
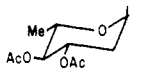
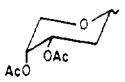
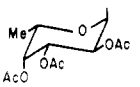
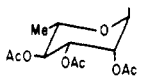
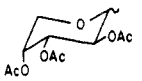
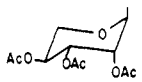
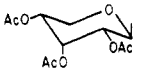
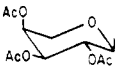
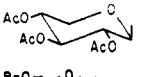
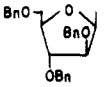
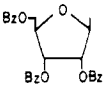
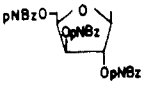
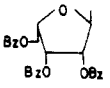
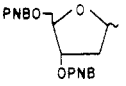
like daunomycinone possesses a number of phenolic hydroxyl groups which under the reaction conditions used are so much less reactive than the secondary hydroxyl group in position 10 that glycosidation in that position is favored in spite of the statistical advantage of the phenolic groups. One difficulty was forseen, namely, that ϵ-rhodomycinone (5) unlike daunomycinone possesses a methyl ester group which would be likely to saponify during the deblocking of the protecting groups attached to the sugars. To avoid this, it was decided to carry out the deblocking using sodium methoxide in absolute methanol.

Henry's glycosidation procedure[8] using mercuric salt was tried on tetra-*O*-acetyl-α-D-glucopyranosyl chloride and bromide, both of which readily gave the 10-substituted glycoside as evidenced by the presence of the three phenolic groups in the NMR spectrum of the blocked glycoside 6. The yield of this product was only 20%, but it was possible to separate the unreacted aglycon from the reaction mixture by chromatography on silica gel and then recycle it to increase the overall yield. The yield of the free glycoside obtained by the saponification with sodium methoxide in absolute methanol was nearly quantitative, and no loss of the methyl ester group attached to position 7 occurred during this reaction.

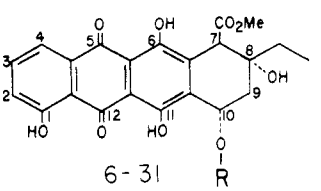
To prepare the target 2-deoxy-L-fucopyranosyl-ϵ-rhodomycinone (23) and 2-deoxy-L-rhamnopyranosyl-ϵ-rhodomycinone (24), we used the 2-deoxy-di-*O*-acetyl-α-L-fucopyranosyl halides and 2-deoxy-di-*O*-acetyl-α-L-rhamnopyranosyl halides recently prepared in our laboratory by treating di-*O*-acetyl-L-fucal and di-*O*-acetyl-L-rhamnal with hydrogen halides.[9] The halides were converted to the blocked glycosides (7 and 8) by reaction

Table I. Blocked Glycosides of ϵ-Rhodomycinone

Compd no.	R	Time of rxn, h	Elution solvent	Yield, %	Mp, °C	Formula	Analyses
6		72	Acetone-petr ether[a] (1:1)	21	250	$C_{36}H_{38}O_{18}$	C, H
7		24	$CHCl_3$	81	142-146	$C_{32}H_{34}O_{14}\cdot 0.5H_2O$	C, H
8		28	$CHCl_3$	83	Amorphous	$C_{32}H_{34}O_{14}\cdot 0.5H_2O$	C, H
9		24	Ether-petr ether[a] (4:1)	75	130-132	$C_{31}H_{32}O_{14}$	C, H
10		72	$CHCl_3$	26	223-225	$C_{34}H_{36}O_{16}\cdot 0.5H_2O$	C, H
11		72	$CHCl_3$	7	210-211	$C_{34}H_{36}O_{16}\cdot 0.5H_2O$	C, H
12		72	$CHCl_3$	26	97-100	$C_{33}H_{34}O_{16}\cdot H_2O$	C, H
13		72	$CHCl_3$	2	232-237	$C_{33}H_{34}O_{16}\cdot 0.5H_2O$	C, H
14		8 days	$CHCl_3$	0.5	124-129	$C_{33}H_{34}O_{16}\cdot 0.5H_2O$	C, H
15		72	$CHCl_3$	24	235-237	$C_{33}H_{34}O_{16}\cdot H_2O$	C, H
16		72	$CHCl_3$	24	232-234	$C_{33}H_{34}O_{16}$	C, H
17		24	Petr ether-acetone (1:1)	23	147-150	$C_{48}H_{46}O_{13}$	C, H
18		96	Petr ether-acetone (1:1)	22	250-254	$C_{48}H_{40}O_{16}\cdot 0.5H_2O$	C, H
19		9 days	$CHCl_3$	13	270-274	$C_{48}H_{37}N_3O_{22}\cdot 0.5H_2O$	C, H, N
20		72	Petr ether-acetone (1:1)	65	Amorphous	$C_{48}H_{40}O_{16}\cdot 0.5H_2O$	C, H
21		15	Petr ether-acetone (1:1)		137-140	$C_{41}H_{34}N_2O_{18}\cdot 0.5H_2O$	C, H, N

[a] Bp 30-60 °C.

with ϵ-rhodomycinone in tetrahydrofuran using mercuric salts as catalysts. The products could be separated from the unreacted sugar and the aglycon on a column of silica gel by elution with chloroform. This separated the unreacted sugar from the desired glycosides. The unreacted aglycon remained on the column and could be recovered by washing with methanol. The yield of 2-deoxy-di-*O*-acetyl-L-fucopyranosyl-ϵ-rhodomycinone (**7**) was above 80%. Deblocking proceeded quite smoothly, giving the desired 2-deoxy-L-fucopyranosyl-ϵ-rhodomycinone (**23**). The position of linkage of the fucopyranosyl residue was established by NMR spectroscopy which revealed three phenolic OH groups in the low-field region of the spectrum,



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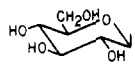
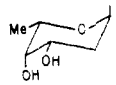
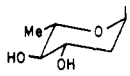
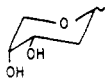
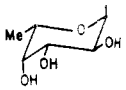
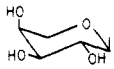
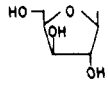
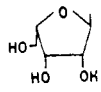
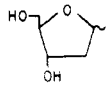
indicating that glycosidation occurred through the secondary hydroxyl group at position 10. The NMR spectrum also showed that the compound was anomerically pure and that it existed in the α-L configuration, which is characterized by a small coupling of the equatorial anomeric proton with the axial and equatorial protons on carbon 2

Table II. Deblocked Glycosides of ϵ-Rhodomycinones

Compd no.	R	Mp, °C	Formula	Analyses
22		259-261	$C_{28}H_{30}O_{14}\cdot 0.5H_2O$	C, H
23		225-227	$C_{28}H_{30}O_{12}\cdot 0.5H_2O$	C, H
24		227-230	$C_{28}H_{30}O_{12}\cdot 0.5H_2O$	C, H
25		200-206	$C_{27}H_{28}O_{12}$	C, H
26		240-242	$C_{28}H_{30}O_{13}\cdot H_2O$	C, H
27		218-222	$C_{27}H_{28}O_{13}\cdot H_2O$	C, H
28		228-238	$C_{27}H_{28}O_{13}\cdot H_2O$	C, H
29		258-262	$C_{27}H_{28}O_{13}\cdot 0.5H_2O$	C, H
30		Amorphous	$C_{27}H_{28}O_{13}$	C, H
31		182-206	$C_{27}H_{28}O_{12}\cdot H_2O$	C, H

of the fucopyranosyl residue.

The preparation of 2-deoxy-di-*O*-acetyl-L-rhamnopyranosyl-ϵ-rhodomycinone (**8**) was achieved in 80% yield, and the saponification to the free glycoside (**24**) was carried out with sodium methoxide.

The third target compound, 2-deoxy-D-ribopyranosyl-ϵ-rhodomycinone (**25**), was obtained by reacting 2-deoxy-D-ribopyranosyl chloride with ϵ-rhodomycinone and saponification of the blocked glycoside **9** with sodium methoxide. The preparation of 2-deoxy-α-D-ribofuranosyl-ϵ-rhodomycinones (**21** and **31**) was carried out in the same way from 2-deoxy-di-*O*-*p*-nitrobenzoylribofuranosyl chloride and ϵ-rhodomycinone.

The synthesis of the 2-hydroxylated glycosides, namely, the L-fucopyranosyl-ϵ-rhodomycinones (**18** and **26**) and the blocked L-rhamnopyranosyl-ϵ-rhodomycinone (**11**), as well as the pentosyl-ϵ-rhodomycinone in the pyranose (**12–16**, **27**) and furanose forms (**17–20** and **28–31**), was carried out by reacting the corresponding glycosyl halide with ϵ-rhodomycinone in tetrahydrofuran in the presence of mercuric salts as a catalyst and deblocking with sodium methoxide. Table I shows the physical constants and analyses of the compounds prepared.

Properties of ϵ-Rhodomycinone Glycosides. The ϵ-rhodomycinone glycosides prepared are red crystalline solids which are often hemi- or monohydrated. A dihydrate has been isolated in the case of the arabinose derivative **26**. However, upon heating under reduced pressure, the compound changed to the monohydrate form. The hue of the red coloration of each of the ϵ-rhodomycinone glycosides differed slightly from one compound to the other. This is probably due to their crystalline forms, since upon dissolution all gave identical UV–visible spectra indistinguishable from that of the aglycon.[10] The infrared spectrum of ϵ-rhodomycinone is also quite similar to that of its glycosides. All show a broad OH band at ν 3400 cm^{-1} and a carbonyl stretching band at ν 1725 cm^{-1} due to the methyl ester group of the aglycon. In the blocked glycosides, this band is broader and larger since it envelops the acetate bands of the sugar residue.

The electron-impact ionization mass spectrum of ϵ-rhodomycinone has been described in the literature.[11] The mass spectra of the glycosides, both blocked and unblocked, show no molecular peaks or peaks resulting from the fragmentation of the glycosides. They show only those fragments originating from the aglycon. The lack of molecular peaks in the compounds prepared is not surprising since the aromatic aglycon is much more stable than the sugar moiety. To observe the molecular peaks in the anthracycline glycosides, chemical ionization is needed.[12]

The NMR spectra of the glycoside acetates were quite useful in confirming the structure of these compounds and showing that the sugar was linked to position 10. This was apparent from the three phenolic protons at low field. The NMR spectra were also useful in establishing anomeric purity and the configuration of the glycosides. The presence of anomeric mixtures could be observed by the broadening of the proton signals or by their doubling. The anomeric configuration in the pure glycosides was established from the coupling constants of the anomeric protons. Thus, for example, the small coupling constant of the anomeric proton of glycoside **7** and the sharpness of the other signals suggested that it existed in a pure α configuration in which the anomeric proton is in an equatorial position. In contrast, the broad signals of

glycoside **9** and the doubling of the signals of glycoside **13** were indicative of anomeric mixtures which were difficult to separate by chromatography. Table I shows the direction of the glycosidic bond of the pure products as deduced from their NMR spectra. The wavy C'-1 bond denotes that the product was isolated as an anomeric mixture.

Screening Results. Both the blocked and unblocked glycosides prepared were subjected to biological testing. The rationale for testing the former compounds was that in the daunorubicin series, antitumor activity is enhanced by lipophilicity.[7] Tests were made at Bristol Laboratories and at the National Cancer Institute. The compounds prepared were tested for antibiotic activity on *Bacillus substilis* which showed activity for the 2-deoxy-di-*O*-acetyl-D-ribopyranosyl-ϵ-rhodomycinone (**9**). However, no induction was observed for any of the glycosides subjected to the ILB test.[12] Antitumor activity on P388 lymphatic leukemia in CDF mice was observed for 2-deoxy-di-*O*-acetyl-D-ribopyranosyl-ϵ-rhodomycinone (**9**) which had a T/C of 125 at a dose of 64 mg/kg/day and 110 at a dose of 128 and 32 mg/kg/day. The unblocked glycoside **25** had a T/C of 115 at a dose of 13 mg/kg/day. 2-Deoxy-L-fucopyranosyl-ϵ-rhodomycinone (**23**) had a T/C of 109 at a dose of 18 mg/kg/day. The fact that the blocked glycoside **9** was active suggests that glycosidation with deoxy sugars may be used to induce activity in anthracycline aglycons. Perhaps attaching 2-deoxy-D-ribose to a more potent aglycon than ϵ-rhodomycinone would yield an anthracycline analogue with desirable properties.

Experimental Section

Melting points were determined with a Köfler block and are uncorrected. Nuclear magnetic resonance spectra were run on a Varian HA-100 spectrometer. Infrared spectra were run on a Perkin-Elmer 621 spectrophotometer. Ultraviolet–visible spectra were recorded by Mr. Thomas Grebinski on a Cary-14 spectrophotometer. Mass spectra were recorded and measured by Mr. M. P. Gilles on a Varian M-66 mass spectrometer. Microanalyses were run in the Department of Chemistry and Chemical Engineering microanalysis laboratory by Mr. M. P. Gilles and Miss P. M. Smiley on a Perkin-Elmer 240 elemental analyzer. Unless indicated otherwise, thin-layer chromatograms were run on Eastman Kodak 13181 silica gel plates and eluted with reagent chloroform as the developing solvent. Preparative chromatography columns were packed with Sargent-Welch SC14608 silica gel (60–200 mesh).

ϵ-Rhodomycinone (5). The ϵ-rhodomycinone was supplied by Bristol Laboratories, a division of Bristol-Myers of Syracuse, N.Y. The ϵ-rhodomycinone was recrystallized from chloroform–cyclohexane until constant melting point (213 °C). The mass spectrum was in agreement with the tabulated data found in the literature.[11]

Synthesis of the Blocked Glycosides (7–21). A suspension of the required glycosyl halide (1 g, 3.7 mmol), ϵ-rhodomycinone (1 g, 2.3 mmol), mercuric bromide (830 mg, 2.3 mmol), mercuric cyanide (60 mg, 230 μmol), and finely ground 3A molecular sieves (5 g) in tetrahydrofuran (55 mL) was stirred and heated under reflux. Two additional amounts of the glycosyl halide (1 g each) were added at regular intervals. After the times listed in Table I, the mixture was allowed to cool and filtered to remove the molecular sieves. The residue was washed well with chloroform and the combined filtrates were evaporated to a syrup under reduced pressure. The syrup was dissolved in chloroform (200 mL) and the chloroform solution washed with a 2 M potassium iodide solution (4 × 175 mL) to remove any mercuric salts. The chloroform solution was dried over sodium sulfate and evaporated to dryness under reduced pressure. The resulting product was dissolved in the elution solvent listed in Table I (30 mL) and applied to a silica gel column (2 × 80 cm) which was eluted with the same solvent. The various fractions collected were tested by TLC on silica gel plates eluted with chloroform. The first fractions were yellow and contained sugar derivatives; the next fractions were red and contained the desired glycoside; later fractions contained mixtures of the glycoside and unreacted aglycons. Evaporation of the pure glycoside fractions yielded crystals of the blocked glycoside (see Table I).

Synthesis of the Deblocked Glycosides (7–21). The blocked glycoside (100 mg) was dissolved in methanol (20 mL) containing an excess of freshly prepared sodium methoxide and stirred at room temperature for 20 min. The purple solution was then poured into a separatory funnel containing a sodium hydrogen sulfate solution (0.5 M, 200 mL) and the resulting emulsion extracted with chloroform (4 × 200 mL). The combined chloroform extracts were dried over sodium sulfate and evaporated to dryness under reduced pressure. The residue was dissolved in hot 95% ethanol (50 mL), filtered, and allowed to evaporate slowly in an open beaker. The crystals were filtered and washed with ether (see Table II).

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