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THE ANTHRACYCLINIC COMPLEX RETAMYCIN, 1. STRUCTURE DETERMINATION OF THE MAJOR CONSTITUENTS

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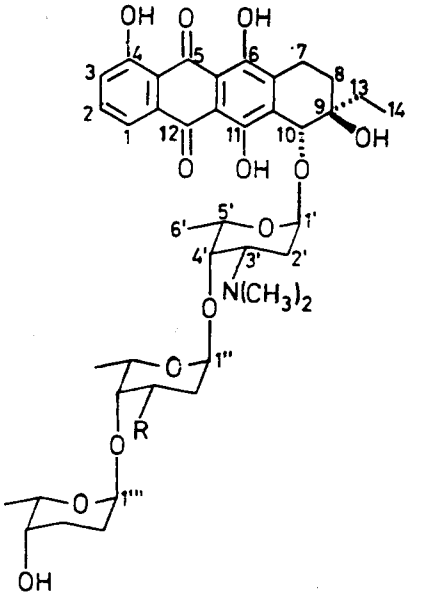
ABSTRACT.—Two major constituents, retamycins E1 [**1**] and E2 [**2**], were isolated from the anthracyclinic complex retamycin. Both compounds are derivatives of γ-rhodomycinone glycosidically linked to rhodosamine. In addition, **1** contains two molecules of rhodinose and **2** one of each rhodinose and deoxyfucose. Composition, sequence, and stereochemical details were determined by high field ^{1}H nmr spectroscopy and fabms. **1** and **2** were found to be identical with rhodilunancins A and B (= cosmomycins A and B).

Retamycin, an anthracyclinic complex produced by *Streptomyces olindensis* (1), belongs to the rhodomycin group (2). Its high antitumor activity against several solid tumors (2,3) incited pharmacological and preclinical investigations (4). First positive results in the treatment of human leukemias (5) prompted a more detailed study of the chemical composition of the complex. In this paper we describe the separation and structure elucidation of two major constituents of retamycin.

RESULTS AND DISCUSSION

Cc of the crude complex produced two main products of slightly different R_f values, designated retamycins E1 [**1**] and E2 [**2**]. Acidic hydrolysis (6) gave the same aglycone, γ-rhodomycinone (7), and the sugars rhodosamine and rhodinose in both cases.

Retamycin E2 [**2**] produced a third sugar identified as 2-deoxyfucose. Partial hydrolysis (8) liberated first rhodinose from both compounds, indicating the terminal position of this sugar. The molecular weights of retamycins E1 [**1**] and E2 [**2**] and the sequence determinations of their constituent sugars were obtained by application of the technique of fabms (9,10). The spectra were run using the bis(2-hydroxy-ethyl)sulfide



1 R=H
2 R=OH

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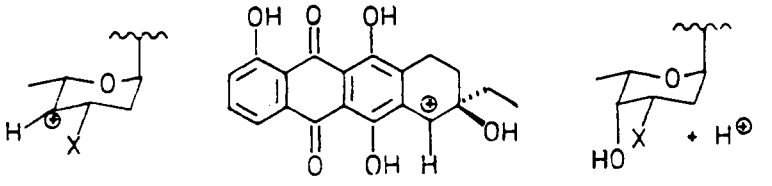
matrix (11) because unreliable results for both the $[MH]^+$ and sequence ions were obtained by using the glycerol matrix (12). Compound **1** produced a strong $[MH]^+$ ion at *m/z* 756, corresponding to a composition of one residue each of γ-rhodomycinone and rhodosamine and two residues of rhodinose. Two kinds of sequence ions, both containing the γ-rhodomycinone moiety, were observed in the spectrum (Figure 1). In one case (type **I'**), 658 and 528 (type **II**) are present in the spectrum, thus providing evidence for the sequence γ-rhodomycinone–rhodosamine–2-deoxyfucose–rhodinose. As expected, pentaacetyl **2** produced a prominent $[MH]^+$ ion at *m/z* 982.

The fragmentation pattern merits a few comments. Most interestingly, the fragmentation leading to ions of type **II** becomes dominant in the presence of a



Type **I**

m/z	X
510	NMe_2
624	H
640	OH

Type **I'**

m/z 353

Type **II**

m/z	X
528	NMe_2
658	OH

FIGURE 1. Sequence ions from retamycins E1 [**1**] and E2 [**2**]. Only the terminal sugar of the fragments is indicated.

fragmentation occurs at the glycosidic linkage level, generating carbenium ions described by structures **I** and **I'** at *m/z* 624, 510, and 353 (the first and the last ones are particularly abundant). Conversely, the ion at *m/z* 528, a very intense peak described by the general structure **II**, carries a charge by virtue of being protonated and is generated by fragmentation between the glycosidic oxygen and C-1 of the sugar, followed by hydrogen rearrangement from the portion lost as neutral moiety. These ions indicate the sequence γ-rhodomycinone–rhodosamine–rhodinose–rhodinose for **1**. The spectrum of the tetraacetyl derivative confirmed these findings by peaks occurring at *m/z* $[MH]^+$ 924, 768, 750, and 654.

Similarly, the fabms of retamycin E2 [**2**] shows an abundant $[MH]^+$ ion at *m/z* 772 relevant to the presence of all three sugars in the molecule. Sequence ions of both types at *m/z* 640 (type **I**) and 353 dimethylamino or hydroxyl function in position 2-*cis* relative to the glycosidic linkage. In the absence of such a function, or in the case of a *trans* relationship, the ions of types **I** and **I'** are produced exclusively.

On the basis of these chemical and spectroscopic findings, we can propose the structures for retamycins E1 [**1**] and E2 [**2**] as shown. The structures are identical with those published for rhodilunancins A and B (13), which in turn have been found to be identical with cosmomycins A and B (14).

Further confirmation of the proposed structures was obtained by high field ^1H nmr spectroscopy. Detailed analysis by multiple decouplings and comparison with data from ciclamycins 4 (15) and 5 (16), which contain the same sugars in comparable positions, allowed the exact assignment of almost all peaks except those in the region between 1.5 and 2.1 ppm corresponding to the positions 2

and 3 of the sugars (Table 1). Chemical shifts and coupling constants confirm all stereochemical features required for structures **1** and **2**, especially the α-glycosidic linkages and the equatorial orientation of the sugar protons in positions 4′, 4″, and 4‴. These assignments together with the physical constants prove conclusively the identity of retamycins E1 and E2 with the rhodilunancins and cosmomycins. As suggested by Li and Chen (13), retamycin E2 or rhodilunancin B may be identical too with rhodomycin Y, described in 1972 without stereochemical details (17). Similarly, retamycin E1 should be identical with the γ-rhodomycinone glycoside obtained in 1975 on hydrogenation of a pentaglycosidic β-rhodomycinone derivative (18).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. ^{1}H nmr spectra were recorded on a Bruker WM 400 spectrometer using $CDCl_3$ as solvent and TMS as an internal standard. Fabms were run in bis(2-hydroxyethyl) sulfide as the matrix on a Kratos MS 80 instrument, equipped with post-acceleration detector and high-field magnet, using a Xenon beam of 6 keV energy and a current of ca. 30 μA. Acetylation was carried out on analytical samples for 36 h, using pyridine-Ac_2O (1:1). Volatiles were evaporated by an N_2 current before taking the mass spectra of the crude acetylation mixture. Acidic hydrolysis and tlc analysis of the sugars were carried out according to Oki and co-workers (6,8).

FRACTIONATION OF RETAMYCIN COMPLEX.—Crude complex (2 g) was fractionated by cc (100 g of Si gel 60), using $CHCl_3$ with increasing amounts (1 to 20%) of MeOH as eluent. Nine fractions (A to I), corresponding to decreasing R_f

TABLE 1. ^{1}H-nmr Chemical Shifts of Retamycins E1 [**1**] and E2 [**2**] (400 MHz, $CDCl_3$, after H/D exchange).

1			**2**		
Position	δ (ppm)	*J* (Hz)	Position	δ (ppm)	*J* (Hz)
6′	1.06 d	7.0	14	0.93 t	7.5
14	1.07 t	7.5	6′	1.00 d	7.0
6″	1.14 d	7.0	6″	1.05 d	7.0
6‴	1.24 d	7.0	6‴	1.12 d	7.0
8a,b 13a,b 2′ax,eq 2″ax,eq 2‴ax,eq 3′ax,eq 3″ax,eq 3‴ax,eq	1.55–2.10 m		8a,b 13a,b 2′ax,eq 2″ax,eq 2‴ax,eq 3′ax,eq 3‴ax,eq	1.40–1.95 m	
NMe	2.20 s		NMe	2.06 s	
7a,b	2.86 m		7a,b	2.75 m	
4‴	3.43 bs		4‴	3.39 bs	
4″	3.55 bs		4″	3.48 bs	
4′	3.74 bs		4′	3.59 bs	
5‴	3.89 q	7.0	5‴ 3″	3.87 q	7.0
5″	4.03 q	7.0	5″	4.03 q	7.0
5′	4.38 q	7.0	5′	4.31 q	7.0
1″	4.78 d	3.0	1″	4.67 d	2.5
1‴	4.91 bs		10	4.81 s	
10	4.94 s		1‴	4.89 d	3.0
1′	5.38 d	3.5	1′	5.26 d	3.5
3	7.26 dd	9.5/1.5	3	7.24 dd	9.5/1.5
2	7.66 t	9.5	2	7.64 t	9.5
1	7.85 dd	9.5/1.5	1	7.82 dd	9.5/1.5

values, were collected. After evaporation, fraction E yielded 460 mg (23%) of a red, amorphous powder.

RETAMYCIN E1 [**1**].—Fraction E (400 mg) was submitted to cc (100 g of Si gel 60), using *n*-BuOH–HOAc (100:1) saturated with H_2O as eluent. Fractions of R_f 0.22 (tlc in the same solvent) yielded, after evaporation and crystallization from CH_2Cl_2/hexane, pure **1** as a red powder with mp 145–148° [lit. (13) 140–142°].

RETAMYCIN E2 [**2**].—Further elution with the same solvent, evaporation of fractions of R_f 0.19 and crystallization from CH_2Cl_2/hexane produced pure **2** as a dark red powder with mp 146–150° [lit. (13) 154–156°].

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