# Ruticulomycins, New Anthracycline Antibiotics

*Sir:*

In the past decade a considerable number of anthracycline antibiotics has been described (1). Most of these compounds are aminoglycosides of various hydroxylated tetrahydronaphthacenequinones differing from one another in the oxygenation pattern of the aromatic rings and in the number and nature of the attached sugars. Because these compounds bear a formal structural resemblance to the tetracycline antibiotics and appear likely to have a similar biochemical origin (2), they have been studied intensively.

The authors present a preliminary report on the isolation of two new anthracycline antibiotics which belong to a spectral class not previously reported. The antibiotics are produced by cultivation of a strain of *Streptomyces rubrireticuli* under conventional conditions of agitation and aeration when grown in a medium composed of 1% molasses, 1% glucose, 1% N-Z Amine Type A,[1] and 0.1% calcium carbonate. Crude mixtures of the antibiotic components were isolated by solvent extraction of the filtered mash at neutral pH values. The organic layers were extracted with dilute mineral acid, the acidic extracts were neutralized, then extracted with chloroform. The chloroform extracts were dried, concentrated to small volume, and diluted with hexane. The precipitated bright-red to orange powder contains numerous minor and two major components which are partially separated by solvent partition chromatography on columns of diatomaceous earth using a system composed of cyclohexane-butanol–water-acetic acid (465:925:1100:13). Approximately equal amounts of fairly pure ruticulomycins A and B were obtained. Final purification was obtained by repartition using mixtures of benzene-petroleum ether–alcohol-water (8:12:15:5 for A, 3:1:3:1 for B). Ruticulomycin A crystallizes from acetone-hexane in dark-red plates, m.p. 183–184° dec., $\lambda_{max.}^{MeOH}$ 235, 258, 295, and 475 mμ ($E_{1cm.}^{1\%}$ 650, 300, 120, and 170, respectively), and significant infrared bands (KBr) at 3450 (hydroxyl), 1740 (ester), 1668, 1624 [hydrogen bonded quinone (3)], and 1576 cm.$^{-1}$. Reproducible analytical figures could only be obtained by drying the compound over $P_2O_5$ for 20 hours at 70° and $10^{-2}$ mm. pressure.

*Anal.*—Found: C, 58.52; H, 6.71; N, 1.80. (O)methyl, 7.26; (N)methyl, 4.42; mol. wt. 942 (thermistor).

Ruticulomycin B crystallizes from acetone-ether in red rosettes, m.p. 179–180° dec. The ultraviolet spectrum is virtually identical with that of A, except for some differences in $E_{1cm.}^{1\%}$ values (690, 300, 120, and 190, respectively), and the infrared spectrum differs from that of A only in minor detail. Although not so hygroscopic as A, B was dried for analysis under the same conditions.

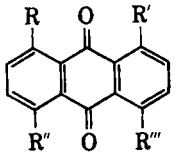
*Anal.*—Found: C, 57.09; H, 6.37; N, 1.84. (O)methyl, 5.87; (N)methyl, 2.13.

Both antibiotics show absorption maxima at 595 and 650 mμ when dissolved in concentrated sulfuric acid.

The number and position of the hydroxyl groups have a marked influence upon the absorption spectra of the various hydroxyanthraquinones (Table I). This fact has been used as a diagnostic tool in elucidating the hydroxylation pattern of the various anthracyclines (1). In this respect it is clear that the spectra of ruticulomycins A and B are closely related to that of 1,4-dihydroxyanthraquinone (Table I, No. *1;* broad visible maximum at 470 mμ which shifts to 545 mμ in alkaline solution). The tetracyclic nature of A and B was demonstrated by zinc dust distillation which produced a hydrocarbon whose ultraviolet absorption spectrum (maxima at 398, 420, 446, and 476 mμ) in chloroform solution was very similar to that of naphthacene (maxima at 397, 419, 444, and 478 mμ).

The common anthracyclines readily undergo acid hydrolysis to produce a mixture of sugars and an insoluble aglycone chromophore. In this respect, ruticulomycins A and B are exceptional, for they do not give rise to a nitrogen-free chromophore except under very vigorous acid conditions (heating in 48% hydrobromic

TABLE I.—VISIBLE MAXIMA OF A VARIETY OF HYDROXYANTHRAQUINONES




| No. | Substance R | R′ | R″ | R‴ | Visible max., mμ | Ref. |
|---|---|---|---|---|---|---|
| *1* | OH | H | OH | H | 470 (MeOH) | (4) |
| *2* | OH | OH | H | H | 430 (MeOH) | (4) |
| *3* | OH | H | H | OH | 420 (MeOH) | (4) |
| *4* | OH | OH | OH | H | 529,516,495,483 (cyclohexane | (5) |
| *5* | OH | OH | OH | OH | 563,548,524,513,490 (cyclohexane) | (6) |

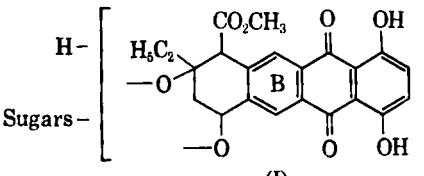
[1] N-Z Amine Type A is an enzymatic digest of casein. Marketed by the Sheffield Chemical Division, National Dairy Products Corp.

acid in glacial acetic acid after acetylation with acetic anhydride/pyridine), and then in very poor yield. Under these strenuous conditions, the anthracycline terminal ring undergoes dehydration to the fully aromatic system. Our amorphous product has so far resisted purification but possesses a visible absorption maximum at 478 mμ in methanol solution. Brockmann has shown that in many cases conversion of anthraquinones to the corresponding tetracenequinones results primarily in an increase in ultraviolet absorption intensity and has relatively little influence upon the position of the visible maxima (7). Aklavinone (II) would appear to be exceptional, for in this case a pronounced wavelength shift was obtained (8). Because this introduces an element of uncertainty in the spectral conclusions, 1,4-dihydroxynaphthacene-5,12-quinone, m.p. 285°, was prepared by the Friedel-Crafts condensation of 1,4-dimethoxybenzene and naphthalene-2,3-dicarboxylic acid anhydride.

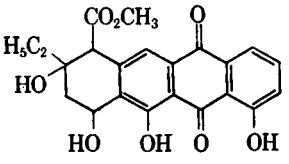
*Anal.*—Calcd. for $C_{18}H_{10}O_4$: C, 74.48; H, 3.47; O, 22.05. Found: C, 74.17; H, 3.68; O, 21.48.

Due to poor methanol solubility, carbon tetrachloride was used as solvent for spectral examination, and a visible maximum was observed at 466 mμ. This is in reasonable agreement with that of 1,4-dihydroxyanthraquinone (max. at 470 mμ). Thus, there seems to be little doubt that the chromophore of the ruticulomycins is as depicted in formula I. These observations serve to emphasize the uniqueness of the ruticulomycins among the anthracyclines, since all of the previously described members have at least one hydroxyl substituent in ring B (1).

Using the anthracycline parent nucleus (1) and placing the ester function by analogy to those compounds of this series whose structures are already known, it seems reasonable to propose structure I as a working hypothesis for further studies and to infer that A and B differ from one another in the sugar portion of the molecule.




(I)




(II)

(1) For a review of these compounds, see Brockmann, H., "Progress in the Chemistry of Organic Natural Products," Vol. 21, Springer-Verlag, Vienna, Austria, 1963, p. 121.
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# Characterization of a New Magnoliaceae Alkaloid

*Sir:*

In a previous communication (1) the authors reported results of preliminary studies on the alkaloids of *Magnolia acuminata* L. Fractionation of the stem extracts revealed the major alkaloids to be of the quaternary type. Five such bases were detected by paper chromatographic technique. Of these, three phenolic alkaloids—*viz.*, salicifoline, magnoflorine, and magnocurarine—were identified. Choline was revealed to be the fourth base. Identification of salicifoline and magnocurarine was confirmed by isolation of the picrates of the alkaloids and comparison with authentic samples.

Chromatography of the purified quaternary alkaloid fraction indicated magnoflorine to be the major phenolic base. While purifying other frac-