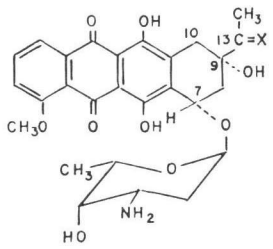
THE IDENTIFICATION OF ε-RHODOMYCINONE AND 7-DEOXY-DAUNORUBICINOL AGLYCONE IN DAUNORUBICIN BEERS

Sir:

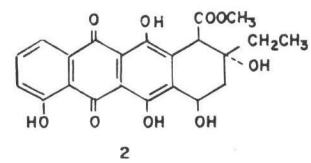
From a soil sample collected in Michaelmas Cay, Australia, we isolated a *Streptomyces* species designated as PD Mycology No. J566. This microorganism was found to produce the important antitumor antibiotic daunorubicin (**1a**) which was earlier isolated and characterized independently as daunomycin by ARCAMONE *et al.*,[1] and as rubidomycin by DUBOST, *et al.*[2] During the course of our isolation studies of J566 beers we noted the presence of red lipophilic components which could be extracted into ethyl acetate or *n*-butanol. Such extracts were diluted with *n*-heptane and basic compounds removed by extraction with dilute acid. The remaining organic layer [OL-1] was concentrated and the residue partitioned between heptane and 9:1 methanol - water. The aqueous methanol layer was back-extracted with heptane and concentrated. Silica gel chromatography of the residue or, in some cases, direct crystallization from methanol afforded significant yields of a deep red compound, mp 220~222°C; $[\alpha]_D$ + 80° (*c* 0.50, $CHCl_3$). Spectral data suggested that this metabolite was ε-rhodomycinone (**2**), an anthracyclinone isolated previously by BROCKMANN and his co-workers.[3] This identity was confirmed by comparing the IR and PMR spectra of our material with the spectra obtained from an authentic sample of ε-rhodomycinone.* After this work was completed, we learned that DI MARCO and ARCAMONE briefly mentioned[4] the isolation of **2** from fermentation beers of *Streptomyces peucetius* which also produces daunorubicin.** The co-production of **1a** and **2** is interesting, especially in view of the recent work of PAULICK, *et al.*,[5] concerning the biosynthesis of daunorubicin.

A second component was isolated from our daunorubicin beers when [OL-1], from above, was extracted twice with 2:1 methanol - water. The red aqueous methanol extracts were combined, back-extracted with heptane, and finally extracted with chloroform. The red chloroform extracts were dried and concentrated. Crystallization of the residue from chloroform-methanol or ethyl acetate-methanol afforded a pure, dark red solid, mp 243~245°C (dec.); $[\alpha]_D$ −128° (*c* 0.22, 1:1 $CHCl_3$ - MeOH). The structure of this compound was shown to be 7-deoxy-daunorubicinol aglycone (**3b**) on the basis of the following data.

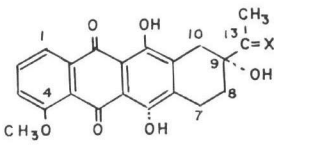
The molecular formula, $C_{21}H_{20}O_7$ (mol. wt. 384.37), of the metabolite was established by elemental analysis and a molecular ion found at 384 in its mass spectrum. The ultraviolet spectrum (λ_{max}^{MeOH} 235, 253, 290, 375, 472, 498, 528 nm) shows that it possesses a hydroxylated anthraquinone system similar or identical to that present in daunomycinone (**1a**, H in place of $C_6H_{12}NO_2$). The absence of infrared absorption near 1710 cm^{-1} precludes the presence of a saturated carbonyl function. The PMR spectrum



1a X = O
1b X = H, OH



2



3a X = O
3b X = H, OH

* We thank Dr. HANS BROCKMANN for supplying us with a sample of ε-rhodomycinone for these comparisons.

** We thank a referee for calling our attention to this paper.

(90 MHz, d_6-DMSO; δ relative to TMS=0) is similar to that of daunomycinone in showing three adjacent aromatic protons as multiplets at 7.75 (2H) and 7.5 (1H), two hydrogen-bonded phenolic hydroxyls at 13.83 and 13.31, an exchangeable tertiary hydroxyl singlet at 4.2, and an aromatic methoxyl at 3.91. Two distinct differences in the non-aromatic proton signals of the metabolite completed its structural chracterization: (1) a -CH(OH)CH_3 function is shown by a methyl doublet at 1.12 coupled to H at 3.55 which is further coupled to an exchangeable hydroxyl proton at 4.62, and (2) a complex pattern corresponding to four benzylic protons appears in the 2.5~2.8 region; another complex AB pattern appears in the 1.5~1.9 region corresponding to a methylene group adjacent to a benzylic methylene and a chiral center.

The above data are consistent with the identification of the metabolite as 7-deoxy-daunorubicinol aglycone (**3b**). The location of the CH(OH)CH_3 and tertiary hydroxyl functions on C-9 (rather than C-8) is more likely considering the source of the metabolite. A chemical synthesis of 7-deoxy-daunorubicinol aglycone (**3b**) was recently reported by T. SMITH, *et al.*[6] We thank Dr. SMITH for comparing our material with his synthetic **3b**. The differences between the two samples could be traced to a difference of optical purity at C-13.

Although daunorubicinol (**1b**) is the major mammalian metabolite of daunorubicin (**1a**), daunorubicinol aglycone and 7-deoxy-daunorubicinol aglycone (**3b**) are two of the several additional metabolites found by TAKANASHI and BACHUR.[7] Recently, WILEY and MARSHALL[8] have reported the bioconversion of daunorubicin to 7-deoxy-daunorubicin aglycone (**3a**) by various microorganisms. After our work was completed, a paper was presented describing the conversion of daunorubicin to **3a** and **3b** by using an enzyme concentrate isolated from *Streptomyces steffisburgensis*.[9]

During our attempts to isolate **3b** it became evident that only a trace amount of this compound is present in our daunorubicin beers which are usually run for 4~6 days with vigorous aeration. However, when these beers are allowed to stand overnight at pH 7.5 (but not at pH 4), without agitation, nearly all of the daunorubicin is destroyed and substantial quantities of **3b** are produced. These "aged" beers proved to be the only practical source of 7-deoxy-daunorubicinol aglycone. The manner in which **3b** is formed concomitantly with the disappearance of daunorubicin suggests that the latter is converted to **3b** by enzymatic transformations, at least one of which is inhibited by oxygen. This same sort of oxygen-sensitivity was noted by BACHUR and GEE[10] in their studies of reductive glycosidic cleavage of daunorubicin by various tissue fractions.

Acknowledgements

We thank the physical chemistry department of Parke, Davis, Ann Arbor, for determination of spectra and Dr. F. A. MACKELLAR for helpful discussions concerning the PMR spectra of various anthracyclinones. We are indebted to Mr. D. MURPHEY for isolating an additional amount of **3b** for further characterization; Mrs. B. GRAHAM and Mrs. L. BALTA for the selection of suitable strains of the J566 culture; and to Mr. M. LENZINI for his assistance in monitoring the various stirred-jar fermentations used in this work.

This work was supported by Contract NO1-CM-43754 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

D. L. KERN
R. H. BUNGE
J. C. FRENCH
H. W. DION

Research Laboratories
Antibiotics & Microbial
Development Section
Parke, Davis & Company
Detroit, Mich. 48232, U.S.A.

(Received November 15, 1977)

References

1) ARCAMONE, F.; G. FRANCESCHI, P. OREZZI, G. CASSINELLI, W. BARBIERI & R. MONDELLI: Daunomycin. I. The structure of daunomycinone. J. Am. Chem. Soc. 86: 5334~5335, 1964
CASSINELLI, G. & P. OREZZI: Daunomycin–isolation and properties of a new antibiotic with antitumor activity. Giorn. Microbiol. 11: 167~174, 1963

2) DUBOST, M.; P. GANTER, R. MARAL, L. NINET, S. PINNERT, J. PREUD'HOMME & G. WERNER:

Rubidomycin, a novel antibiotic with cytotoxic properties. C. R. Acad. Sci. Paris 257: 1813~1815, 1963

3) BROCKMANN, H.; H. BROCKMANN, Jr. & J. NIEMEYER: The absolute configuration of anthracyclinones. Tetrahedron Letters 1968: 4719~4724, 1968

4) DI MARCO, A. & F. ARCAMONE: DNA complexing antibiotics: daunomycin, adriamycin and their derivatives. Arzneimitt. Forsch. 25: 368~375, 1975

5) PAULICK, R.; M. CASEY & H. WHITLOCK: A ^{13}C nuclear magnetic resonance study of the biosynthesis of daunomycin from $^{13}CH_3{}^{13}CO_2Na$. J. Am. Chem. Soc. 98: 3370~3371, 1976

6) SMITH, T.; A. FUJIWARA, D. HENRY & W. LEE: Synthetic approaches to adriamycin. J. Am. Chem. Soc. 98: 1969~1971, 1976

7) TAKANASHI, S. & N. BACHUR: Daunorubicin metabolites in human urine. J. Pharmacol. Exp. Ther. 195: 41~49, 1975

8) WILEY, P. & V. MARSHALL: Microbial conversion of anthracycline antibiotics. J. Antibiotics 28: 838~840, 1975

9) MARSHALL, V.; E. REISENDER, L. REINEKE & P. WILEY: Daunomycin metabolism by *Streptomyces steffisburgensis*. 16th Interscience Conference on Antimicrobial Agents and Chemotherapy: Abstract 48; October 27~29, 1976; see J. Antibiotics 29: 966~968, 1976

10) BACHUR, N. & M. GEE: Microsomal reductive glycosidase. J. Pharmacol. Exp. Ther. 197: 681~686, 1976