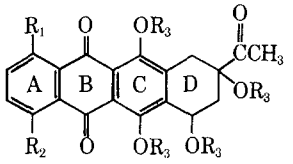
Identity of Rubidomycin and Daunomycin

Sir:

Rubidomycin (1), an antibiotic isolated from the culture of *Streptomyces coeruleorubidus*, has been reported (2) to be chemically identical to daunomycin, an antibiotic isolated from the mycelium of *Streptomyces peucetius*. This identity was based on comparisons of ultraviolet and infrared data, chromatographic behavior, and certain color reactions. Similar biological activities have also been reported (3) for these two antibiotics. The structures of daunomycinone and daunosamine, the aglycone and the sugar moiety, respectively, of daunomycin, were elucidated by Arcamone and co-workers (4). In view of the current interest in rubidomycin and daunomycin as antitumor agents, it was deemed essential to place this identity on a more rigorous chemical basis.

Commercial samples[1] of the two antibiotics showed diffuse and indistinct X-ray diffraction patterns; the recrystallized[2] samples yielded dim, but definitely nonequivalent, patterns. The commercial samples showed similar, but definitely not identical, spectra (infrared, ultraviolet, and NMR), although samples which were exhaustively exchanged with D_2O yielded identical NMR spectra. By thin-layer chromatography, commercial daunomycin was shown to be homogeneous and commercial rubidomycin was shown to be heterogeneous, consisting of one major component (chromatographically identical with daunomycin) contaminated by a minor component possessing the same color and R_f value as the aglycone.[3] Because these antibiotics are hygroscopic and labile, they are difficult to characterize *per se;* therefore a common crystalline derivative for these samples was sought. Since the objective of this investigation was to show the identity of these two antibiotics, and since their elemental data indicated different amounts of water, it was necessary to purify these samples by a common procedure. To remove the aglycone from the rubidomycin sample and to start with materials of the same degree of hydration in the characterization studies, samples of both antibiotics were partitioned between chloroform and water. The aqueous solutions were lyophilized to yield solids that were chromatographically homogeneous. Benzoylation of these materials was conducted under identical conditions with identical stoichiometry. The use of excess benzoyl chloride in pyridine at room temperature for 20 hr. gave mixtures of benzoates consisting primarily of a pentabenzoate. This major component was isolated by preparative thin-layer chromatography and then was recrystallized from ethanol. The NMR of the crystalline solid showed that it was a pentabenzoate, and the infrared spectrum identified it as the tetra-*O*-mono-*N*-benzoate (I),[4] m.p. 236–238°. The equivalence of the two pentabenzoates from the two antibiotics was demonstrated by experimentally identical infrared, ultraviolet, NMR, chromatographic, melting point, and elemental data, as well as by X-ray diffraction patterns, which were identical in spacings and intensities.[5] The crude benzoylation products from the respective antibiotics showed very similar chromatographic patterns. When the major components were isolated from preparative plates, the mixture from rubidomycin yielded 58% pentabenzoate, 11% tetrabenzoates,[6] and 15% of an unknown benzoate(s), and the mixture from daunomycin yielded 56% pentabenzoate, 13% tetrabenzoates,[6] and 16% of the same unknown benzoate(s) isolated from the rubidomycin mixture. The essential identity of the benzoylation yield data and the identity of the pentabenzoates from the two antibiotics provide proof of the identity of these two materials.



I

R_1R_2 = H, OCH_3
R_3 = three of the R_3 are Bz and the fourth is daunosamine, *N,O*-dibenzoate

(1) Dubost, M., Ganter, P., Maral, R., Ninet, L., Pinnert, S., Preud'homme, J., and Werner, G.-H., *Compt. Rend.*, **257**, 1813(1963).
(2) Cassinelli, G., and Orezzi, P., *Giorn. Microbiol.*, **11**, 167(1963).
(3) Price, K. E., Buck, R. E., and Lein, J., in "Antimicrobial Agents and Chemotherapy—1964," Sylvester, J. C., ed., American Society for Microbiology, Ann Arbor, Mich., 1965, p. 511.

[1] Samples of daunomycin, a product of Farmitalia, Milan, Italy, and rubidomycin, a product of Rhone-Poulenc Co., Vitry sur Seine, France, were generously furnished by Cancer Chemotherapy National Service Center.

[2] The authors thank Dr. W. Yanko, Monsanto Chemical Co., for the recrystallization procedure.

[3] Mild acid hydrolysis of the antibiotics afforded the same aglycone, daunomycinone, in good yields. The equivalence of these aglycones was established by X-ray diffraction patterns, which were identical in spacings and intensities.

[4] The locations of the methoxyl in ring A and the glycosidic linkage in the molecule have not been established. The latter problem is presently under investigation in this laboratory.

[5] The samples which yielded these X-ray patterns were recrystallized from a chloroform–ethanol mixture.

[6] More than one tetrabenzoate is theoretically possible. Chromatography of the isolated tetrabenzoate fraction showed one main component and 2 trace contaminants.

(4) Arcamone, F., Franceschi, G., Orezzi, P., Cassinelli, G., Barbieri, W., and Mondelli, R., *J. Am. Chem. Soc.*, **86**, 5334, 5335(1964).

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Errata

In the article titled "New Compounds: Synthesis of Some Sulfazopyridines for Pharmacological Study" (1), the first author's name, page 1358, should read:

Hassan Y. Aboul-Enein

(1) Aboul-Enein, H. Y., Khalifa, M., and Abou-Zeid, Y. M., *J. Pharm. Sci.*, **56**, 1358(1967).

In the article titled "Irreversible Enzyme Inhibitors LXXXII. Candidate Active-Site-Directed Irreversible Inhibitors of Dihydrofolic Reductase VII. Derivatives of 2,4-Diaminopyrimidine I" (1), the following correction should be made:

In Table I on page 568 under "% Inactivation" the number opposite compound XII should read "0."

(1) Baker, B. R., Jackson, G. D. F., and Meyer, R. B., Jr., *J. Pharm. Sci.*, **56**, 566(1967).

In the Book Review titled "Nonionic Surfactants" (1), the following correction should be made on page 664:

Reviewed by James Swarbrick
School of Pharmacy
University of Connecticut
Storrs, Conn.

(1) Swarbrick, J., *J. Pharm. Sci.*, **56**, 663(1967).

In the article titled "Acetylation of Acetaminophen in Tablet Formulations Containing Aspirin" (1), the following correction should be made in Table II, page 1120:

Under "DAPAP, mg./Tab.," between 4.13 and 36, insert "DAPAP, mcg./Tab."

(1) Koshy, K. T., Troup, A. E., Duvall, R. N., Conwell, R. C., and Shankle, L. L., *J. Pharm. Sci.*, **56**, 1117(1967).

In the article titled "Diffusion of Drugs Across the Isolated Mesentery" (1), *References 8* and *11*, page 469, should read:

(8) Engle, D., and Kerekes, A., *Klin. Wochschr.*, **5**, 1709(1926); through *Chem. Abstr.*, **21**, 133(1927).
(11) Brodie, B. B., Burns, J. J., Mark, L. C., Lief, P. A., Bernstein, E., and Papper, E. M., *J. Pharmacol. Exptl. Therap.*, **109**, 26(1953).

(1) Shenouda, L. S., and Mattocks, A. M., *J. Pharm. Sci.*, **56**, 464(1967).

In the article titled "Radiopharmaceuticals" (1), the following corrections should be made:

1. On page 1, column 1, the first footnote paragraph should read:

Received from the Department of Pharmaceutical Chemistry, School of Pharmacy, University of Southern California, Los Angeles, CA 90007, and the *Radioisotope Research Laboratory, Veterans Administration Center and Department of Radiology, School of Medicine, University of California at Los Angeles, Los Angeles, CA 90024

2. On page 5, *Footnotes 1* and *2* should read:

[1] Marketed as Hippuran-131 by Abbott Laboratories, North Chicago, Ill., as Hipputope by E. R. Squibb & Sons, New York, N. Y., and as Hippuran I 131 by Nuclear Consultants, Chicago, Ill.
[2] Marketed as Ethiodol-131 by Abbott Laboratories, North Chicago, Ill.

3. On page 14, column 2, line 32, ^{99m}Te should read ^{99m}Tc.

(1) Wolf, W., and Tubis, M., *J. Pharm. Sci.*, **56**, 1(1967).

In the article titled "Pemoline and Magnesium Hydroxide *Versus* Pemoline: Enhancement of Learning and Memory of a Conditioned Avoidance Response in Rats" (1), the following corrections should be made:

1. On page 290, *Footnote 1* should read:

[1] Tradenamed as Cylert by Abbott Laboratories, North Chicago, Ill. Abbott-30400; an equimolar combination of 2-imino-5-phenyl-4-oxazolidinone (Abbott-13397) and magnesium hydroxide.

2. On page 291, *Footnote b* in Table II should read:

[b] Mean jump-out time significantly different from controls p 0.05 (10).

(1) Plotnikoff, N., and Meekma, P., Jr., *J. Pharm. Sci.*, **56**, 290(1967).