apomorphine. Striatal tissue was obtained from male rats weighing 200–225 g. The incubation mixture consisted of 15 mM Tris-HCl buffer, pH 7.5, containing 1 mM EDTA and 0.01% ascorbic acid, and the final volume was 1.0 mL. The concentration of the striatal tissue was 2.5 mg (wet weight) and the final concentration of [^{3}H]NPA (specific activity 58.8 Ci/mmol) was 0.50 nM. Incubations were carried out at 25 °C for 20 min. Nonspecific binding was determined in the presence of 1 μM *d*-butaclamol.

Determination of the Striatal Concentrations of DA, DOPAC, HVA, and 6,7-Dihydroxy-3-chromanamine. Female rats (180–200 g) were injected ip with a dose of 100 μmol/kg of 6,7-dihydroxy-3-chromanamine dissolved in physiological saline solution containing sodium metabisulfite (0.1%) as an antioxidant. After periods of 15 and 45 min, the animals were sacrificed, the brains were rapidly removed, and the striatal and cerebellar tissues were dissected and immediately frozen on dry ice and stored at −80 °C. DA, DOPAC, and HVA in the corpus striatum were determined by reversed-phase HPLC (Nucleosil 5 C_{18}) with amperometric detection after isolation on Sephadex G-10 columns according to the method of Westerink and Mulder.[24] The cerebellar tissues of control rats were used as blanks and as tissue for recovery experiments. The recoveries were 86–89% and the values were not corrected for recovery.

The striatal concentrations of 6,7-dihydroxy-3-chromanamine following the administration of 100 μmol/kg, ip were determined after 15 and 45 min by using a previously reported HPLC/electrochemical detection method for 6,7-ADTN.[19] Slight modifications of the mobile phase, i.e., 0.1 M phosphate–acetate buffer, pH 4.5, were found to be necessary. Concentrations were calculated with the aid of a standard. Recoveries were estimated by assaying spiked brain samples from untreated rats. The recovery was 78.8 ± 3.8% (n = 5), mean ± SD.

Determination of the Partition Coefficients of 1a and 1b. The partition coefficients (log p values) in an octanol–phosphate buffer system (pH 7.4) were determined according to the method of Feenstra et al.[25] using the above analytical methodology.

Registry No. **1a**, 53463-78-8; **1b**, 90990-80-0; **2**, 64173-08-6; **3**, 64139-42-0; **4**, 90990-81-1; **5**, 90990-82-2; dopamine, 51-61-6; 6,7-dimethoxy-4-chromanone oxime, 64139-41-9.

(24) Westerink, B. H. C.; Mulder, T. B. A. *J. Neurochem.* **1981**, *36*, 1449.

(25) Feenstra, M. G. P.; Homan, J. W.; Everts, R.; Rollema, H.; Horn, A. S. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, in press.

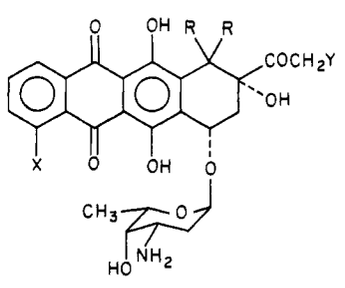
Total Chemical Synthesis and Antitumor Evaluation of 4-Demethoxy-10,10-dimethyldaunomycin

Jose Alexander,† Ish Khanna,† Daniel Lednicer,‡ Lester A. Mitscher,*† Tarik Veysoglu,† Zbigniew Wielogorski,† and Richard L. Wolgemuth‡

Department of Medicinal Chemistry, Kansas University, Lawrence, Kansas 66045, and Research Laboratories, Adria Laboratories, Columbus, Ohio 43216. *Received January 3, 1984*

The novel anthracycline analogue 4-demethoxy-10,10-dimethyldaunomycin was prepared in nine chemical steps from 5,8-dimethoxy-2-tetralone. It proved to be inactive as an antitumor agent in the mouse P388 lymphocytic leukemia model.

Synthetic studies on anthracycline antitumor antibiotics such as daunomycin (**1**) and doxorubicin (**2**) have been pursued intensively.[1,2] Present interest is turning increasingly to total synthesis of unnatural analogues inaccessible from fermentation-derived substrates in the hope of producing drugs with significantly altered biological properties.[1-6] In this context we report here our experiences in the total synthesis and antitumor evaluation of 4-demethoxy-10,10-dimethyldaunomycin (**3**).



1, X = OCH_3; R = Y = H
2, X = OCH_3; R = H; Y = OH
3, X = Y = H; R = CH_3

Previous knowledge of structure–activity relationships at the C-10 position of anthracycline antibiotics is relatively sparse. Natural congeners are limited to those possessing a C-10β-CO_2CH_3[7-11] and C-10β-OH[12,13] moiety. These are known to retain significant antitumor activity, but they have not been commercialized. From chemical transformation of daunomycin and doxorubicin, it is known that dehydration produces $\Delta^{9,10}$-analogues that proved to be toxic to mice and did not cure their tumors (L1210).[4,14,15]

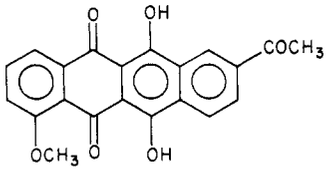
† Kansas University.
‡ Adria Laboratories.

(1) Arcamone, F. "Doxorubicin Anticancer Antibiotics"; Academic Press: New York, 1981.
(2) El Khadem, E., Ed. "Anthracycline Antibiotics"; Academic Press: New York, 1982.
(3) Mosher, C. W.; Wu, H. Y.; Fujiwara, A. N.; Acton, E. M. *J. Med. Chem.* **1982**, *25*, 18.
(4) Potti, G.; Israel, M. *J. Med. Chem.* **1982**, *25*, 478.
(5) Penco, S.; Angelucci, F.; Arcamone, F.; Ballabio, M.; Barchielli, G.; Franceschi, G.; Franchi, G.; Suarto, A.; Vanotti, E. *J. Org. Chem.* **1983**, *48*, 405.
(6) Lown, J. W.; Sondhi, S. M.; Mandal, S. B.; Murphy, J. *J. Org. Chem.* **1982**, *47*, 4304.
(7) Strelitz, F.; Flon, H.; Weiss, U.; Asheshov, I. N. *J. Bacteriol.* **1956**, *72*, 90.
(8) Brockmann, H.; Lenk, W. *Chem. Ber.* **1959**, *92*, 1904.
(9) Richle, W.; Winkler, E. K.; Hawley, D. M.; Dobler, M.; Keller-Schierlein, W. *Helv. Chim. Acta* **1972**, 55, 467.
(10) Oki, T.; Matsuzawa, Y.; Yoshimoto, A.; Kamata, N.; Kitamura, I.; Hori, S.; Takamatsu, A.; Umezawa, M.; Ishizuka, M.; Naganawa, H.; Suda, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1975**, *28*, 830.
(11) Nettleton, D. E.; Bradner, W. T.; Bush, J. A.; Coon, A. B.; Moseley, J. E.; Myllmaki, R. W.; O'Herron, F. A.; Schreiber, R. H.; Vulcano, A. L. *J. Antibiot.* **1977**, *30*, 525.
(12) Brockmann, H.; Waehneldt, Th. *Naturwissenschaften* **1961**, *48*, 717.
(13) Brockmann, H.; Waehneldt, Th.; Niemeyer, J. *Tetrahedron Lett.* **1969**, 415.

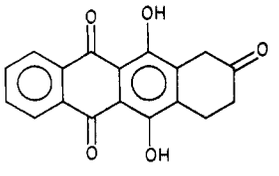
0022-2623/84/1827-1343$01.50/0

In vitro activity against human lymphoblastic leukemia cells (CCRF-CEM) was also significantly less in tissue culture. A C-10-monomethyl-$\Delta^{9,10}$-anhydrodaunomycin derivative is inactive in mice bearing a P388 tumor at 100 mg/kg.[17] Of the two epimeric C-10-monomethoxy analogues, the C-10β-epimer was the more active in vivo (P388).[16] The C-10-monomethyl analogues of daunomycin and doxorubicin of uncertain stereochemistry were found to be as efficatious as daunomycin but were only a tenth as potent. This substance, however, also lacked a C-9-acyl side chain.[17]

It is clear from these considerations that some leeway is available for modification of the C-10 position with retention of bioactivity. We decided, therefore, to synthesize the C-10,10-dimethyl analogue because of these and the following considerations. One of the major biotransformation pathways of anthracycline antibiotics involves reduction of the ketonic moiety of the C-9 side chain.[18,19] The same C-9 side chain reputedly plays a significant role in stabilizing intercalations through hydrogen bonding. A relatively bulky C-10,10-dimethyl group would be expected to have a significant inhibitory effect on either or both of these important processes. Further, chemical bisdehydration of the aliphatic ring (to analogues of 4) would be prevented, leading to greater chemical stability toward acidic reagents during chemical synthesis.

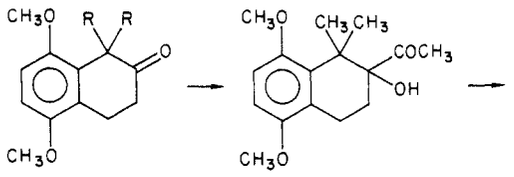


4



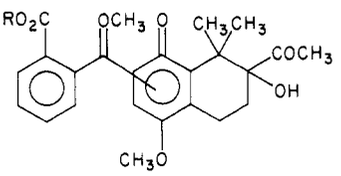
5

Chemistry. Initial studies of the direct methylation of ketone **5**, a well-known anthracycline synthon,[20,21] were frustrated by its relatively poor solubility in common organic solvents, making large-scale work difficult. Thus we turned to methylation of decalin **6**[22] instead. Conversion of the resulting dimethyl analogue to the α-hydroxy methyl ketone **8** using the lithium salt of methyl vinyl ether proceeded, as expected, dramatically more efficiently (80% yield without recycling) than with the corresponding β-tetralone because of the absence of highly acidic hydrogens at C-1. The α-hydroxy ketone moiety survived Friedel–Crafts acylation with phthalic acid monomethyl ester, demonstrating a surprisingly strong inductive effect. After saponification, the intermediate substituted benzophenones (**9b**) cyclized efficiently wih concomitant ether cleavage to give the desired substituted tetrahydronaphthacenequinone **10** under the influence of BCl_3. Introduction of the missing C-7 α-hydroxyl group using a well-known route involving benzylic halogenation and

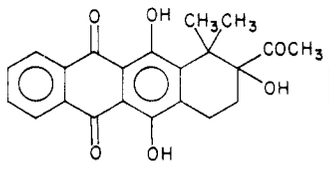


6, R = H
7, R = CH_3

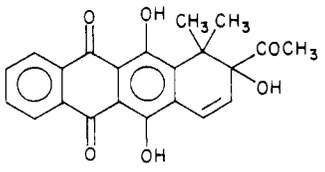
8



9a, R = CH_3
b, R = H

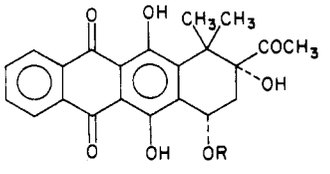


10

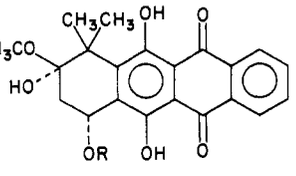


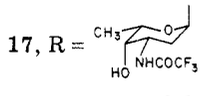
13

solvolysis[20] proved very difficult to do in this series on a significant (50 mg) scale. The molecule was very prone to dehydrohalogenation (to **13**) under most reaction conditions. This result was very disappointing for it obviated some of the anticipated advantages of working in the C-10 dimethyl series. Fanatic adherence to the conditions specified in the Experimental Section ultimately produced the desired alcohol, after equilibration, in usable yield (30% overall from **10**). The stereochemistry at C-7 H in **11** and **12** was assigned on the basis of a comparison of the

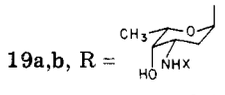


11, OR = α-OH
12, OR = β-OH

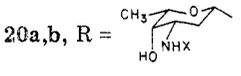




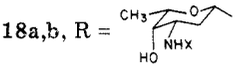
17, R =



19a,b, R =



20a,b, R =



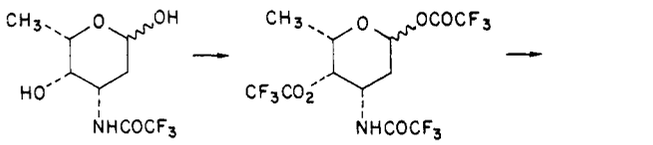
18a,b, R =

a, X = $COCF_3$; b, X = H

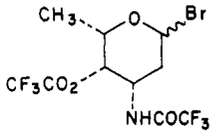
a, X = $COCF_3$; b, X = H

corresponding NMR values with those of daunomycinone and 7-epidaunomycinone ($\nu_{1/2}$ = 8 Hz in **11** and 17 Hz in **12**, respectively).[20,23,24] The resulting aglycon (±)- **11** was converted to a separable mixture of all four possible protected glycosides with use of optically active daunosamine, suitably protected and activated, and silver triflate as condensing agent.[27] Thus, the sugar served also as a resolving agent. For this purpose, the *N,O*-trifluoroacetyl derivative of daunosamine **15** was converted to its C-1 bromo analogue **16** (3:1 anomeric ratio) by reaction with

(14) Zunino, F.; DiMarco, A.; Zaccara, A. *Chem. Biol. Interact.* **1979**, *24*, 217.
(15) Arlandini, E.; Vigevani, A.; Arcamone, F. *Farmaco, Ed. Sci.* **1980**, *35*, 65.
(16) Penco, S.; Gozzi, F.; Vigevani, A.; Ballabio, M.; Arcamone, F. *Heterocycles* **1979**, *13*, 281.
(17) Penco, S.; Angelucci, F.; Gozzi, F.; Franchi, G.; Gioia, B.; Vigevani, A.; Arcamone, F. *Int. Symp. Chem. Nat. Prod., 11th, Golden Sands, Bulg.* **1978**, *4*, Part 1, 448.
(18) Bachur, N. R. *J. Pharmacol. Exp. Ther.* **1971**, *177*, 573.
(19) Ahmed, N. K.; Felsted, R. L.; Bachur, N. R. *Biochem. Pharmacol.* **1978**, *27*, 2713.
(20) Kende, A. S.; Tsay, Y.; Mills, J. E. *J. Am. Chem. Soc.* **1976**, *98*, 1967.
(21) Lee, W. W.; Martinez, A. P.; Smith, T. H.; Henry, D. W. *J. Org. Chem.* **1976**, *41*, 2296.
(22) Alexander, J.; Mitscher, L. A. *Tetrahedron Lett.* **1978**, 3403.
(23) Wong, C. M.; Popien, D.; Schwenk, R.; TeRaa, J. *Can. J. Chem.* **1971**, *49*, 2712.
(24) Smith, T. H.; Fujiwara, A. N.; Henry, D. W.; Lee, W. W. *J. Am. Chem. Soc.* **1976**, *98*, 1969.
(25) Smith, T. W.; Fujiwara, A. N.; Lee, W. W.; Wu, H. Y.; Henry, D. W. *J. Org. Chem.* **1977**, *42*, 3653.
(26) Gillard, J. M.; Israel, M. *Tetrahedron Lett.* **1981**, 513.
(27) Arcamone, F.; Penco, S.; Redaelli, S.; Hanessian, S. *J. Med. Chem.* **1976**, 19, 1424.



14 15



16

trimethylsilyl bromide.[26] Isomers **17** and **19a** were identified as α-anomers in part by their ^{1}H NMR peaks at δ 5.48 (dd, J = 2, 3.5 Hz) + 5.15 and 5.43 (dd, J = 2.0, 3.0 Hz) + 5.30 (C-1′ H and C-7 H, respectively); the β-isomer **20a** had corresponding peaks at δ 5.12 (dd, J = 3, 7 Hz) + 5.05. These data agree with literature values for the NMR peaks of anomeric protons of anthracycline glycosides.[28-30] Axial anomeric protons appear as a pair of doublets ($J_{1'ax,2'eq}$ = ~2 Hz, $J_{1'ax,2'ax}$ = ~9 Hz) in β-isomers, whereas in α-isomers the anomeric protons appear as a double doublet (J = ~1.5 and ~3 Hz). Moreover, the chemical shift for C-1′ H is at higher field values in β-isomers (δ 4.9) as compared with that of the α-isomers (α 5.5).[29] The remaining isomer was isolated in very small quantity and was assigned its stereochemistry primarily by difference. In addition, the CD spectra of the four subsequently deblocked isomers were consistent with the assigned stereochemistry by comparison with those of doxorubicin. On deprotection, the signals broadened but were sufficient to demonstrate that the sugars had remained attached. [Prominent signals were seen for the aglycon (CMe signals at δ 1.3 and 1.7; COMe at δ 2.4, 4 × Ar H from δ 7.40–8.65) and for the attached daunosaminyl moiety (methyl doublet at δ 1.25).]

Biological Results. The new glycoside **3** corresponding to daunorubicin was tested intraperitoneally against P388 acute lymphocytic leukemia in mice under conditions under which doxorubicin gave outstanding protection (T/C > 316 at 10 mg/kg). This response is higher than usual (T/C = 250) with this dose. None of the doses of 10,10-dimethyl glycoside were active (T/C = 85–93 up to 10 mg/kg). This result is all the more disappointing and convincing considering that 4-demethoxy analogues are known to be several fold more potent in such tests when compared with the natural antibiotics. Insufficient drug was available to allow determination of an end point so it is possible that **3** is weakly active. However, no activity was observed at doses up to 100 times that of a doxorubicin dose that gave a T/C of 135.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus or on a Fisher-Johns hot stage and are uncorrected. IR spectra were recorded with a Beckman 33 or a Perkin-Elmer 727 infrared spectrophotometer. UV spectra were obtained with a Cary 219 spectrophotometer. ^{1}H NMR spectra were obtained in $CDCl_3$ with a Varian EM360 or FT-80A spectrometer using tetramethylsilane as internal standard. Mass spectral analyses were conducted on a Riber 10-10 or a MAT CH5 mass spectrometer at an ionizing voltage of 70 eV. Microanalyses were performed on a Hewlett-Packard 185B instrument and the results are within ±0.4% where indicated by symbols for the elements of the theoretical values. Analytical thin-layer chromatography was conducted with E. Merck silica gel 60 F-254 precoated plates or with Brinkmann Instruments Sil G/UV-254 precoated plastic sheets. Preparative-layer chromatography was carried out on E. Merck 20 × 20 × 0.25 cm silica gel 60 F-254 or Analtech silica gel GF plates.

5,8-Dimethoxy-1,1-dimethyl-2-tetralone (7). NaH (9.5 g, 50% dispersion in mineral oil) was washed with hexane (2 × 300 mL) and suspended in freshly distilled dimethoxyethane (800 mL) under N_2 atmosphere. The suspension was cooled to 0–5 °C and an excess of CH_3I (22 mL) was added. To the well-stirred mass there was added 5,8-dimethoxy-2-tetralone (**6**: 23 g, 0.11 mol) in DME (750 mL) over a 40-min period. The color of the reaction mixture turned grey. After 3 h at 0–5 °C, the reaction was quenched by the addition of cooled glacial acetic acid (45 mL) over 10–15 min. The solvent was evaporated at reduced pressure and the residue was diluted with water (500 mL) and then extracted with ether (1200 mL). The ether extracts were combined and washed sequentially with aqueous solutions of sodium bisulfite (400 mL), sodium bicarbonate (350 mL), water (200 mL), and brine (250 mL). After drying over anhydrous sodium sulfate, the solvent was removed by distillation. The resulting thick yellowish liquid was adsorbed on silica gel and chromatographed with use of pentane and then pentane–EtOAc (9:1) to give in succession 1.2 g (4.3%) of 5,8-dimethoxy-1,1,3-trimethyl-2-tetralone [mp 101 °C; IR (KBr) 1715, 1600, 1482, 1372, 1352 cm^{-1}; ^{1}H NMR δ 1.15 (3 H, d, J = 6 Hz, $COCHCH_3$), 1.48 (3 H, s, CH_3), 1.53 (3 H, s, CH_3), 2.2–3.3 (3 H, m, $COCHCH_2$), 3.80 (6 H, s, 2 × OCH_3), 6.71 (2 H, s, Ar H); MS, m/z 248 (M^+, 100%); anal. ($C_{15}H_{20}O_3$) C, H], 11.6 g (44%) of 5,8-dimethoxy-1,1-dimethyl-2-tetralone (**7**) [mp 95–96 °C; IR (KBr) 1715, 1598, 1598, 1370, 1340 cm^{-1}; ^{1}H NMR δ 1.53 (6 H, s, $C(CH_3)_2$, 2.47–3.17 (4 H, m, Ar CH_2CH_2), 3.83 (6 H, s, 2 × OCH_3), 6.76 (2 H, s, 2 × Ar H); MS, m/z 234 (M^+, 100); anal. ($C_{14}H_{18}O_3$) C, H], and 4 g (16%) of 5,8-dimethoxy-1-methyl-2-tetralone [mp 91–92 °C; IR (KBr) 1715, 1600, 1482, 1372, 1328 cm^{-1}; ^{1}H NMR 1.33 (3 H, d, J = 7 Hz, $CHCH_3$), 2.4–3.6 (5 H, m, $CHCOCH_2CH_2$), 3.80 (6 H, s, 2 × OCH_3), 6.73 (2 H, s, Ar H); MS, m/z 220 (M^+, 100%); anal. ($C_{13}H_{16}O_3$) C, H]. When 5,8-dimethoxy-1-methyl-2-tetralone, obtained as above, was resubjected to the methylation conditions, a mixture of 37% of **7** and 13% additional trimethyl analogue was obtained.

2-Acetyl-5,8-dimethoxy-1,1-dimethyl-2-hydroxy-1,2,3,4-tetrahydronaphthalene (8). Methyl vinyl ether (15 g, 0.25 mol) was condensed into a flask purged with dry nitrogen and cooled to −40 °C. THF (200 mL) was added under N_2 and the whole cooled to −70 °C. *N,N,N′,N′*-Tetramethylethylenediamine (10 mL) was added to the stirred mass and was followed by the addition over a 5-min period of *t*-BuLi (100 mL, 1.6 mol in pentane). The initial greenish-yellow solution (at −70 °C) turned colorless above −20 °C. The time taken for the temperature to rise to −10 °C was about 1 h. The reaction mixture was recooled to −70 °C and 11.6 g of **7** in 300 mL of THF was added over a 25-min period. The reaction warmed to 0 °C in 1 h and was stirred at 0–5 °C for 2.5 h more before quenching with 350 mL of cold aqueous NH_4Cl. The organic layer was separated and the aqueous layer was extracted with ether, dried, and evaporated. The residual yellowish semisolid was suspended in a cooled (5 °C) mixture of 70% $HClO_4$ (100 mL) and CH_3OH (100 mL), the mixture allowed to warm to room temperature and stirred for 2.5 h, and the product precipitated by adding crushed ice. The filtered residue was suspended in hexane–EtOAc (10:3, 100 mL) and filtered to give 11 g (80%) of pure **8**: mp 165–166 °C; IR (KBr) 3530, 1700, 1600, 1470, 1440, 1370, 1300 cm^{-1}; ^{1}H NMR δ 1.36 (6 H, s, 2 × CCH_3), 1.80–2.10 (2 H, m, Ar CH_2CH_2), 2.25 (3 H, s, CH_3CO), 2.70–2.85 (2 H, m, Ar CH_2), 3.75 (6 H, s, 2 × OCH_3), 6.70 (2 H, s, Ar H); MS, m/z 278 (M^+), 235 ($M^+ - COCH_3$, 100%), 220 (235 − CH_3), 217, 202, etc.; anal. ($C_{16}H_{22}O_4$) C, H.

4-Demethoxy-7-deoxy-10,10-dimethyldaunomycinone (10). A mixture of **8** (5.6 g, mol) and phthalic acid monomethyl ester (20 g, 0.11 mol) in trifluoroacetic anhydride (35 mL) was immersed in an oil bath preheated to 60 °C. The solution was refluxed for 26 h under an N_2 atmosphere, the solvent was removed under reduced pressure, and the thick brownish liquid was diluted with saturated aqueous sodium carbonate solution (250 mL) and ex-

(28) Arcamone, F.; Cassinelli, G.; Franceschi, G.; Orezzi, P. *Tetrahedron Lett.* **1968**, 3353.
(29) Arcamone, F.; Penco, S.; Vigevani, A.; Radaelli, S.; Franchi, G. *J. Med. Chem.* **1975**, *18*, 703.
(30) Umezawa, H.; Takahashi, Y.; Kinoshita, M.; Naganawa, H.; Tatsuta, K.; Takeuchi, T. *J. Antibiot.* **1980**, *33*, 1581.

tracted with chloroform (250 mL). The organic layer was washed successively with aqueous sodium carbonate (4 × 250 mL) and water (2 × 250 mL) and dried, the solvent evaporated, and the residue adsorbed on silica gel. Elution with hexane–EtOAc (10:3) gave a mixture (6.5 g, 75%) of the isomeric intermediate keto monophthalic esters **9a**: IR (KBr) 3430, 1730, 1660, 1600 cm^{-1}; ^1H NMR δ 1.44 (3 H, s, CCH_3), 1.73 (3 H, s, CCH_3), 1.70–2.20 (2 H, m, Ar CH_2CH_2), 2.40 (3 H, s, $COCH_3$), 2.7–3.1 (2 H, m, Ar CH_2), 3.26 (1 H, s, OH), 3.63 (3 H, s, CO_2CH_3), 3.73 (3 H, s, OCH_3), 3.85 (3 H, s, OCH_3), 6.93 (1 H, s, Ar H), 7.4–7.9 (4 H, m, Ar H); MS, m/z 422 ($M^+ - H_2O$), 379 (422 – $COCH_3$); MS (CI, CH_4), 423 ($MH^+ - H_2O$); anal. ($C_{25}H_{28}O_7$) C, H. These were dissolved in MeOH (250 mL) and 8% aqueous NaOH (250 mL) and stirred at 65–70 °C for 24 h. After cooling, the MeOH was removed under reduced pressure and the aqueous solution was cooled and acidified (HCl) to pH 4 and exhaustively extracted with $CHCl_3$. Evaporation gave the isomeric keto acids **9b** (4.3 g) as a brownish fluffy solid: IR (KBr) 3450, 1710, 1595 cm^{-1}; ^1H NMR δ 1.41 (6 H, s, 2 × CH_3), 1.7–2.2 (2 H, m, Ar CH_2CH_2), 2.30 (3 H, s, $COCH_3$), 2.6–3.1 (2 H, m, Ar CH_2CH_2), 3.36 (1 H, br s, OH), 3.60 (3 H, s, OCH_3), 3.73 (3 H, s, OCH_3), 6.89 (1 H, s, Ar H), 7.4–7.9 (4 H, m, Ar H); MS (CI, CH_4), m/z 427 (MH^+); anal. ($C_{24}H_{26}O_7$) C, H.

Acids **9b** were cyclized without further purification by cooling to −78 °C and treatment over 10–15 min with BCl_3 (70 mL, 1 M solution in CH_2Cl_2). The solution was stirred under N_2 at −78 °C for 2 h and then at room temperature for 40 h. After evaporation, the residue was treated with saturated aqueous Na_2CO_3 and then acidified with 2 N HCl to pH 6. The resulting solution was extracted with $CHCl_3$, washed with H_2O, dried, concentrated onto silica gel, and chromatographed over a column of silica gel impregnated with 4% NaH_2PO_4, eluting with C_6H_5–EtOAc (80:1) to give reddish orange quinone **10** (1.23 g): mp 257–258 °C; IR (KBr) 3460, 1690, 1620, 1580 cm^{-1}; ^1H NMR δ 1.55 (3 H, s, CH_3), 1.60 (3 H, s, CH_3), 1.8–2.3 (2 H, m, Ar CH_2CH_2), 2.35 (3 H, s, $COCH_3$), 2.95 (1 H, d, J = 6 Hz, Ar CH), 3.07 (1 H, d, J = 6 Hz, Ar CH), 3.80 (1 H, s, OH), 7.7–7.85 (2 H, m, Ar $H_{2,3}$), 8.2–8.4 (2 H, m, Ar $H_{1,4}$), 13.57 (1 H, s, Ar OH), 14.48 (1 H, s, Ar OH); MS, m/z 380 (M^+); anal. ($C_{22}H_{20}O_6$) C, H.

(±)-4-Demethoxy-10,10-dimethyldaunomycinone (11). Synthetic deoxy aglycone **10** (100 mg, 0.26 mol) was dissolved in CCl_4 (120 mL) by warming at 55 °C with a General Electric 110-125V (275 W) sun lamp. *N*-Bromosuccinimide (52 mg, 0.29 mol) was added and the temperature was maintained between 52 and 55 °C. Close adherence to these conditions was essential. The reaction was monitored by TLC (silica gel impregnated with KH_2PO_4 (C_6H_6–$(CH_3)_2CO$, 12:1) and was stopped when the concentration of a purple product, subsequently shown to be olefin **13**, began to increase. The reaction mixture was filtered and evaporated, and the residue was chromatographed on silica gel impregnated with 4% KH_2PO_4 using C_6H_6 and then C_6H_6–$(CH_3)_2CO$ (100:3), to afford, in order, olefin **13** [mp 213–215 °C; IR (KBr) 3450, 1700, 1622, 1590 cm^{-1}; ^1H NMR δ 1.44 (3 H, s, CH_3), 1.50 (3 H, s, CH_3), 2.10 (3 H, s, $COCH_3$), 4.57 (1 H, s, OH), 5.75 (1 H, d, J = 11 Hz, Ar CH=CH), 7.10 (1 H, d, J = 11 Hz, Ar CH=CH), 7.65–7.85 (2 H, m, Ar $H_{2,3}$), 8.10–8.35 (2 H, m, Ar $H_{1,4}$); MS, m/z 378 (M^+), 335 (378 – $COCH_3$, 100%); anal. ($C_{22}H_{18}O_6$) C, H], recovered starting material (**10**, 21 mg, 21%), undesired racemic epimer **12** [44 mg, 53%; mp 168–170 °C dec; IR (KBr) 3595, 1710, 1630, 1590 cm^{-1}; ^1H NMR 1.45 (3 H, s, CH_3), 1.65 (3 H, s, CH_3), 1.8–2.6 (2 H, m, Ar CH_2CH_2), 2.40 (3 H, s, $COCH_3$), 3.90 (1 H, s, OH), 4.23 (1 H, s, OH), 5.10–5.35 (1 H, m, $\nu_{1/2}$ = 17 Hz, Ar CHOH), 7.65–7.90 (2 H, m, Ar $H_{2,3}$) 8.15–8.35 (2 H, m, Ar $H_{1,4}$), 14.0 (1 H, s, Ar OH), and 14.3 (1 H, s, Ar OH); MS, m/z 396 (M^+), 3.78 (396 – H_2O), 335 (378 – $COCH_3$, 100%); anal. ($C_{22}H_{20}O_7$) C, H], desired racemic epimer **11** [7 mg, 9%; mp 163–166 °C dec; IR (KBr) 3600, 3500, 1710, 1625, 1590 cm^{-1}; ^1H NMR 1.40 (3 H, s, CH_3), 1.65 (3 H, s, CH_3), 1.8–2.6 (2 H, m, Ar CH_2CH_2), 2.40 (3 H, s, $COCH_3$), 3.85 (1 H, m, OH), 4.46 (1 H, s, OH), 5.15 (1 H, m, $\nu_{1/2}$ = 8 Hz, Ar CHOH), 7.65–7.90 (2 H, m, Ar $H_{2,3}$), 8.15–8.35 (2 H, m, Ar $CH_{1,4}$), 14.0 (1 H, s, Ar OH), 14.28 (1 H, s, Ar OH); MS, m/z 396 (M^+), 335 (396 – H_2O – $COCH_3$, 100%); anal. ($C_{22}H_{20}O_7$) C, H.

Equilibration of Racemic 4-Demethoxy-10,10-dimethyl-7-epidaunomycinone (12). Trifluoroacetic acid (5 mL) was cooled to −55 °C and to the slush that formed in 3 min, epimer **12** (52 mg) was added, and the temperature was allowed to rise to room temperature over 40 min. The reaction was allowed to stir for 4 h under N_2 and evaporated at room temperature. The vacuum-dried product was dissolved in $(CH_3)_2CO$ and cooled to 0–5 °C and treated with 4 mL of cold aqueous NH_4OH (formed from 5 mL of concentrated NH_4OH, 10 mL of distilled H_2O, and 10 mL of $(CH_3)_2CO$). After 3 h, $(CH_3)_2CO$ (15 mL) was distilled off at room temperature and the residue drowned with ice. The cold solution was extracted with $CHCl_3$–CH_3OH (4:1, 3 × 50 mL), and the combined organic extracts were evaporated under reduced pressure. The residue was chromatographed over NaH_2PO_4-impregnated silica gel, using C_6H_6–$(CH_3)_2CO$ (100:3) to give 15.8 mg of racemic epimer **11** (30%) and 7.1 mg of recovered racemic **12** (14%).

***N,O,O*-Tris(trifluoroacetyl)daunosamine (15).**[29] *N*-(Trifluoroacetyl)daunosamine (**14**, 90 mg)[25] was suspended in 5 mL of dry ether and stirred at 0–5 °C under N_2. Trifluoroacetic anhydride (3 mL) was added dropwise. After stirring for 2 h, the reaction was allowed to warm to room temperature and stirred for another 8 h. After removal of the solvent under reduced pressure, the residue was stirred at room temperature for 20 min in 40 mL of CCl_4. Filtration and drying gave 146 mg (91%) of **15** as a white, amorphous solid: mp 128–130 °C dec; IR (KBr) 1790, 1695, 1550 cm^{-1}; ^1H NMR δ 1.15 (3 H, d, J = 7 Hz, CH_3), 2.05–2.25 (2 H, m, CH_2), 4.10–4.65 (2 H, m, H_3 and H_5), 5.35 (1 H, m, H_4), 6.05 (1 H, m, H_1), 6.40 (1 H, s, NH); MS, m/z 322 ($M^+ - CF_3CO_2$), 209 (322 – $NHCOCF_3$), 95 (209 – $OCOF_3$); anal. ($C_{12}H_{10}F_6NO_6$) C, H.

***N,O*-Bis(trifluoroacetyl)daunosaminyl Bromide (16).** *N,O*-Bis(trifluoroacetyl)daunosamine (**15**; 40 mg, 0.009 mol) was dissolved in 5 mL of $CHCl_3$ and cooled to 0–5 °C. Bromotrimethylsilane (19 μL, 0.0135 mol) was added while the stirred mass was coming to room temperature (in about 30 min). After 2 h of stirring, the solvent was removed under reduced pressure and the residue was vacuum dried to produce a 100% yield of **16** pure enough, if not allowed to stand too long, for the next reaction: ^1H NMR δ 1.22 (3 H, d, J = 7 Hz, CH_3), 2.05–2.50 (2 H, m, CH_2, H-2), 4.10–4.95 (2 H, m, H_3 + H_5), 5.35 (1 H, m, H_4), 6.10 and 6.45 (1 H, m, anomeric Hs, 3:1), 6.68 (1 H, s, NH).

Isomeric *N*-(Trifluoroacetyl)-4-demethoxy-10,10-dimethyldaunomycins. Racemic aglycon **11** (48 mg) and bromo sugar **16** (freshly obtained from 85 mg of **15** as just described) were dissolved in 15 mL of CH_2Cl_2, 300 mg of 3A molecular sieves were added, and the flask was wrapped in aluminum foil. Silver triflate (85 mg) in 3 mL of Et_2O was added over 5 min. After 30 min, 30 mL of saturated $NaHCO_3$ was added, the aqueous layer was extracted three times with 40 mL of CH_2Cl_2 and the combined organic layers were evaporated. The residue was dissolved in 20 mL of MeOH and the solution refluxed 30 min before filtering while hot. The residue was washed with hot MeOH, and the filtrates were combined and evaporated. TLC (SiGel/C_6H_6–Me_2CO, 12:3) showed the presence of all four possible diastereoiosmers (R_f 0.42, 0.34, 0.24, and 0.21). Chromatography on 4% KH_2PO_4-impregnated silica gel, eluting with C_6H_6–Me_2CO (4:1), separated the four isomers. Pure samples were isolated by PLC (C_6H_6–Me_2CO, 12:3) and were identified in order of elution as the following.

Recovered aglycon **11** (17 mg, 35%).

N-(Trifluoroacetyl)-7,9,1′-*epi*-4-demethoxy-10,10-dimethyldaunomycin (**20a**) (4 mg, 8%): ^1H NMR δ 1.27 (3 H, d, J = 6 Hz, CCH_3), 1.35 (3 H, s, CCH_3), 1.75 (3 H, s, CCH_3), 1.45–1.70 (2 H, m, C-2′ CH_2), 1.80–2.40 (2 H, m, C-8 CH_2), 2.40 (3 H, s, $COCH_3$), 3.30–3.75 (1 H, m, C-4′ H), 3.50 (1 H, s, C-4′ OH), 3.80–4.25 (2 H, m, C-3′ and C-5′ H), 4.40 (1 H, s, C-9 OH), 5.05 (1 H, dd, W_H = 6 Hz, C-7 H), 5.12 (1 H, dd, J = 3, 7 Hz, C-1′ H), 6.70 (1 H, d, NH), 7.65–7.85 (2 H, m, C-2,3 H), 8.15–8.35 (2 H, m, Ar 1,4-H), 13.65 (1 H, s, Ar OH), 14.25 (1 H, s, Ar OH); anal. ($C_{30}H_{30}F_3NO_{10}$) C, H, N.

***N*-(Trifluoroacetyl)-4-demethoxy-10,10-dimethyldaunomycin (17)** (10 mg, 13%): ^1H NMR δ 1.25 (3 H, d, J = 6 Hz, CCH_3), 1.40 (3 H, s, CCH_3), 1.82 (3 H, s, CCH_3), 1.50–1.70 (2 H, m, C-2′ CH_2), 1.85–2.60 (2 H, m, C-8 CH_2), 2.43 (3 H, s, $COCH_3$), 3.35 (1 H, s, C-4′ OH), 3.65 (1 H, m, C-4′ H), 4.00–4.30 (2 H, m, C-3′ and C-5′ H), 4.35 (1 H, s, C-9 OH), 5.15 (1 H, dd, W_H = 7.5 Hz, C-7 H), 5.48 (1 H, dd, J = 2, 3.5 Hz, C-1′ H), 6.60 (1 H, d, J = 8 Hz, NH), 7.70–7.85 (2 H, m, C-2 and C-3 H), 8.20–8.45 (2 H, m, C-1 and C-4 H), 13.65 (1 H, s, Ar OH), 14.25

(1 H, s, Ar OH).

***N*-(Trifluoroacetyl)-7,9-*epi*-4-demethoxy-10,10-dimethyldaunomycin (19a)** (6 mg, 8%); ^{1}H NMR δ 1.28 (3 H, d, J = 6 Hz, CCH_3), 1.35 (3 H, s, CCH_3), 1.85 (3 H, s, CCH_3), 1.45–1.70 (2 H, m, C-2′ CH_2), 1.85–2.40 (2 H, m, C-8 CH_2), 2.37 (3 H, s, $COCH_3$), 3.40 (1 H, s, C-4′ OH), 3.60 (1 H, m, C-4′ H), 4.05–4.45 (2 H, m, C-3′ and C-5′ H), 4.57 (1 H, s, C-9 OH), 5.30 (1 H, dd, W_H = 6 Hz, C-7 H), 5.43 (1 H, dd, J = 2, 3 Hz, C-1′ H), 6.60 (1 H, d, J = 9 Hz, NH), 7.70–7.85 (2 H, m, C-2 and C-3 H), 8.20–8.40 (2 H, m, C-1 and C-4 H), 13.65 (1 H, s, Ar OH), 14.25 (1 H, s, Ar OH).

***N*-(Trifluoroacetyl)-1′-*epi*-4-demethoxy-10,10-dimethyldaunomycin (18a)** (1 mg, 2%). This substance was characterized as its cleavage product due to its small quantity.

Isomeric 4-Demethoxy-10,10-dimethyldaunomycins. The individual *N*-(trifluoroacetyl) glycosides were deblocked by cooling their THF solutions to 0–5 °C and bringing the pH to 13–14 through slow addition of 0.1 N NaOH. After the solutions were stirred for 16 h at 0 °C, the pH was adjusted to 8.0–8.5 by careful addition of 0.1 N HCl, and the solutions were extracted with CH_2Cl_2. The glycosides were purified by individual evaporation and passage over silica gel columns impregnated with 4% KH_2PO_4 using $CHCl_3$–MeOH–HOH (8:2:0.3) for elution. Yields were approximately 80%. Each glycoside was converted to its HCl salt by adding a stoichiometric quantity of MeOH–HCl in MeOH at 0 °C. After stirring 20 min, the salts were precipitated in 90% yield by addition of dry Et_2O.

4-Demethoxy-10,10-dimethyldaunomycin (3): mp 174–6 °C; HCl salt mp, 165–166 °C; UV λ_{max} (MeOH) 254 nm (ε 33 280), 286 (7600), 463 (7910), 492 (9340), 525 (6050); IR (KBr) 3500 cm^{-1}, 1700, 1625; ^{1}H NMR δ 1.25 (3 H, d, J = 6 Hz, CCH_3), 1.30 (3 H, s, CCH_3), 1.70 (3 H, s, CCH_3), 1.50–1.70 (2 H, m, C-2′ CH_2), 1.80–2.60 (2 H, m, C-8 CH_2), 2.40 (3 H, s, $COCH_3$), 3.30–4.70 (5 H, m, C-3′, C-4′, and C-5′ H and C-4′ and C-9 OH), 5.20–5.45 (2 H, m, C-7 and C-1′ H), 7.40–8.20 (2 H, m, C-2 and C-3 H), 8.30–8.65 (2 H, m, C-1 and C-4 H); CD (MeOH) $[\theta]_{287}$ −1.22 × 10^4.

7,9-*epi*-4-Demethoxy-10,10-dimethyldaunomycin (19b): mp 173–176 °C; HCl salt mp 138–140 °C; UV λ_{max} (MeOH) 253 nm (ε 23 790), 285 (6250), 462 (5530), 493 (6630), 527 (4720); IR (KBr) 3500 cm^{-1}, 1710, 1630; ^{1}H NMR δ 1.23 (3 H, s, CCH_3), 1.27 (3 H, s, CCH_3), 1.70 (3 H, s, CCH_3), 1.20–2.30 (4 H, m, C-2′ and C-8 CH_2), 2.30 (3 H, s, $COCH_3$), 3.30–4.40 (5 H, m, C-3′, C-4′, C-5′ H and C-4′ and C-9 OH), 5.10–5.30 (2 H, m, C-7 and C-1′ H), 7.50–7.80 (2 H, m, C-2 and C-3 H), 7.90 and 8.30 (2 H, m, C-1 and C-4 H); CD (MeOH) $[\theta]_{287}$ 0.86 × 10^4.

7,9,1′-*epi*-4-Demethoxy-10,10-dimethyldaunomycin (20b): mp 187–193 °C; HCl salt mp 152–153 °C; UV_{max} (MeOH) 253 nm (ε 30 700), 287 (7690), 463 (7270), 490 (8500), 525 (6000); IR (KBr) 3500 cm^{-1}, 1700, 1625; ^{1}H NMR δ 1.23 (3 H, s, CCH_3), 1.30 (3 H, s, CCH_3), 1.75 (3 H, s, CCH_3), 1.20–2.20 (4 H, m, C-2′ and C-8 CH_2), 2.40 (3 H, s, $COCH_3$), 3.25–4.00 (5 H, m, C-3′, C-4′, and C-5′ H and C-4′ and C-9-OH), 4.80–5.10 (2 H, m, C-1′ and C-7 H), 7.50–7.80 (2 H, m, C-2 and C-3 H), 7.90–8.40 (2 H, m, C-1 and C-4 H); CD $[\theta]_{287}$ 0.78 × 10^4.

1′-*epi*-4-Demethoxy-10,10-dimethyldaunomycin (18b): amorphous; HCl salt mp 142–144 °C.

Testing in Mice against the P388 Lymphocytic Leukemia Model. Doxorubicin and the glycoside hydrochloride **3** were dissolved in saline (Cremophor:saline for the analogue) to final concentrations of 0.01, 0.033, 0.1, 0.33, and 1.0 mg/mL. Female CDF_1 and DBA_2 mice (Laboratory Animal Supply Co., Indianapolis, IN) were fed Purina Laboratory Chow and water ad lib. and adapted to their cages for at least 1 week before use. The tumor was maintained by continuous passage in DBA_2 mice. On day 0, ascitic fluid was removed, diluted with Hawk's balanced salt solution, and counted, and 10^6 cells were implanted ip in a total volume of 0.2 mL. Twenty-four hours later, drug was given ip to groups of five mice for each dilution. The mice were observed for 30 days and T/C (percent) values were determined from the survival rate as compared to the controls. In this test, the lowest dose of doxorubicin (0.1 mg/kg) had a T/C value of 135 and 10 mg/kg of doxorubicin had a T/C value grater than 316. The analogue **3** ranged from 85 to 93 with doses from 0.1 mg/kg to 10 mg/kg. Insufficient drug was available for titrations, but this T/C values is considered to be insignificant.

Acknowledgment. The authors at Kansas University are grateful to Adria Laboratories for generous financial support of this work and all authors are grateful to Dr. H. J. Eichel for support and encouragement.

Registry No. **3**, 91003-74-6; **3**·HCl, 91108-53-1; **6**, 37464-90-7; **7**, 91003-61-1; **8**, 91003-64-4; **9a** (isomer 1), 91003-66-6; **9a** (isomer 2), 91003-67-7; **9b** (isomer 1), 91003-68-8; **9b** (isomer 2), 91003-69-9; **10**, 91003-65-5; **11**, 91003-70-2; **12**, 91003-72-4; **13**, 91003-71-3; **14**, 52471-40-6; **15**, 91108-50-8; **16**, 90146-27-3; **17**, 91003-73-5; **18a**, 91108-52-0; **18b**, 91108-56-4; **18b**·HCl, 91176-63-5; **19a**, 91108-51-9; **19b**, 91108-54-2; **19b**·HCl, 91176-61-3; **20a**, 91109-35-2; **20b**, 91108-55-3; **20b**·HCl, 91176-62-4; 5,8-dimethoxy-1,1,3-trimethyl-2-tetralone, 91003-62-2; 5,8-dimethoxy-1-methyl-2-tetralone, 91003-63-3; methyl vinyl ether, 107-25-5.

Synthesis and Antiarrhythmic and Parasympatholytic Properties of Substituted Phenols. 2.[1] Amides

David M. Stout,*† W. L. Matier, Cynthia Barcelon-Yang,† Robert D. Reynolds,‡ and Barry S. Brown‡

Department of Pharmaceutical Research, American Critical Care Division, American Hospital Supply Corporation, McGraw Park, Illinois 60085. *Received November 9, 1983*

Thirty amides patterned after the antiarrhythmic drug changrolin were synthesized and their antiarrhythmic and parasympatholytic activities were assessed. There was no correlation between antiarrhythmic and parasympatholytic activities. Several of the amides were found to be potent antiarrhythmic agents that possessed low parasympatholytic activity. All of the compounds appear to act by a class I mechanism.

The term "arrhythmia" encompasses a variety of cardiac disorders including rhythm irregularities, increased or decreased frequency of beats, and abnormalities in the propagation of beats. It is therefore not surprising that the drugs available for treating arrhythmias cover a broad spectrum, both structurally and in their mechanisms of action. Unfortunately, all antiarrhythmic drugs also have unwanted side effects, most notably cardiotoxicity, gastrointestinal complications, and adverse CNS effects. Because of the complex nature of arrhythmias and the deleterious side effects of known antiarrhythmic agents, the search for drugs that work by novel mechanisms

† Section of Medicinal/Organic Chemistry.
‡ Section of Pharmacology.

(1) For part 1, see: Stout, D. M.; Matier, W. L.; Barcelon-Yang, C.; Reynolds, R. D.; Brown, B. S. *J. Med. Chem.* **1983**, *26*, 808.

0022-2623/84/1827-1347$01.50/0