Preparation of 3-(Ethoxycarbonyl)-2-(2-furyl)-4-quinolone (III). The anilinomalonate IIc (3.2 g, 10 mmol) was heated with stirring in an oil bath at 250 °C until the mixture had solidified completely. The reaction mixture was cooled to 160 °C and dissolved in 10 mL of dimethylformamide. The solution was cooled to room temperature. Filtration with suction gave 2.4 g (85%) of III: mp >250 °C, TLC; R_f 0.72 (9:1 chloroform–methanol); ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3 H, J = 7.32 Hz), 4.31 (q, 2 H, J = 7.32 Hz), 6.80 (dd, 1 H, J = 1.46, 3.66 Hz), 7.32 (d, 1 H, J = 3.66 Hz) 7.40–8.25 (total 5 H); IR (Nujol) 1725 (s), 1635 (m), 1610 (m), 1570 (s), 1510–40 (s), 1400–1000 (several strong bands); MS m/e calcd for $C_{16}H_{13}NO_4$ 283.0844, M^+ 283.0849 (35), 238 (18), 237 (100).

Preparation of 5,10-Dihydro-11*H*-indeno[1,2-*b*]quinoline-10,11-dione (IVa). Polyphosphoric acid (50 g) was heated to 210 °C in an oil bath, and the β-anilinomalonate IIa (3.42 g, 10 mmol) was added to the hot solution. The mixture was heated for 5 min with stirring at 210–230 °C and was then poured into hot water (150 mL). The solution was cooled to room temperature. Filtration with suction and recrystallization from dimethylformamide gave 1.9 g (75%) of IVa: mp >250 °C (lit.[9] mp 350 °C); TLC R_f 0.54 (9:1 chloroform–methanol); ^{1}H NMR (DMSO-d_6) δ 7.25–8.30 (m, total 8 H); IR (Nujol) 1710–1695 (several strong bands), 1630 (s), 1580 (s), 1540 (s), 760 (s), 710 (s); MS m/e calcd for $C_{16}H_9NO_2$ 247.0633, M^+ 247.0639 (100), 248 (18), 219 (14), 190 (17).

Preparation of 5,10-Dihydro-4*H*-cyclopenta[*b*]thieno-[6,5-*b*]quinoline-4,5-dione (IVb). Utilization of the above described cyclization procedure on 2.5 g of IIb gave 0.9 g (50%) of IVb: mp >250 °C; TLC R_f 0.47 (9:1 chloroform–methanol); ^{1}H NMR (DMSO-d_6) δ 7.29 (d, 1 H, J = 4.39 Hz), 7.35–7.78 (m, 3 H, J = 8.06, 4.39 Hz), 7.90 (d, 1 H, J = 5.13 Hz), 8.20 (d, 1 H, J = 8.06 Hz); IR (Nujol) 1700 (s), 1635 (s), 1570 (s), 1530 (s), 1435 (w), 1355 (w), 1230–1170 (weak bands), 835–625 (several strong bands); MS m/e calcd for $C_{14}H_7NO_2S$ 253.0197, M^+ 253.0237 (100), 254 (17), 196 (12).

Preparation of 5,5-Dimethyl-3-(ethoxycarbonyl)-4-hydroxy-2(5*H*)-furanone (Va). 2-Bromo-2-methylpropanoyl chloride (5.6 g, 30 mmol) was added slowly with stirring and cooling (cold water bath) to diethyl (ethoxymagnesio)malonate[10] (30 mmol) in 50 mL of toluene. The mixture was stirred for 2 h at room temperature and was then acidified with 2 M hydrochloric acid. The toluene phase was dried with sodium sulfate and concentrated on a rotary evaporator to give the crude product (8 g) that was used without further purification.

The crude product was dissolved in 70 mL of water containing 3.7 g (26 mmol) of potassium carbonate, and the mixture was heated until the evolution of carbon dioxide had subsided. The mixture was acidified with hydrochloric acid and extracted with chloroform. The chloroform phase was dried with sodium sulfate and concentrated on a rotary evaporator to give 4.4 g (72%) of the product that solidified with time: mp 83–84 °C; TLC R_f 0.35 (8:2 chloroform–methanol); ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3 H, J = 7.08 Hz), 1.59 (s, 6 H), 4.42 (q, 2 H, J = 7.08 Hz), 8.90 (s, 1 H); IR (Nujol) 1760 (s), 1720 (w), 1610 (s), 1315 (s), 1200–800 (several strong bands); MS calcd for $C_9H_{12}O_5$ 200.0684, M^+ 200.0695 (24), 156 (83), 155 (23), 154 (36), 142 (12), 139 (21), 138 (20), 126 (12), 114 (43), 86 (38), 69 (11), 67 (16), 59 (100), 58 (48), 55 (11), 45 (46).

Preparation of the Crude 4-Chloro-5,5-dimethyl-3-(ethoxycarbonyl)-2(5*H*)-furanone (Vb). The hydroxybutenolide Va (4.4 g) was dissolved in 20 mL of phosphorus oxychloride, and diisopropylethylamine (2.8 g) was added. The mixture was refluxed for 3 h, and the excess of phosphorus oxychloride was then removed with an evaporator. The residue was extracted three times with ether, and the combined ether layers were concentrated on a rotary evaporator to give 3.5 g (75%) of Vb: ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3 H, J = 7.08 Hz), 1.62 (s, 6 H), 4.40 (q, 2 H, J = 7.08 Hz); MS calcd for 218.0345, M^+ 218.0354 (14).

Preparation of 4-(3,4-Dimethoxyanilino)-5,5-dimethyl-3-(ethoxycarbonyl)-2(5*H*)-furanone (Vc). The chlorobutenolide Vb (1.32 g, 6 mmol), 3,4-dimethoxyaniline (1.02 g, 6 mmol), and 0.73 g of triethylamine were heated at 90 °C in an oil bath over night (12 h) with efficient stirring. The solution was cooled to room temperature, and 50 mL of chloroform was added. The chloroform solution was washed twice with water, dried with anhydrous sodium sulfate, and concentrated on a rotary evaporator. Methanol (10 mL) was added to the crude product, and the mixture was placed in a refrigerator to give the product, 0.84 g (41%): mp 145–146 °C; TLC R_f 0.17 (6:1 benzene–EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.4 (s + t, total 9 H), 3.88 (s, 3 H), 3.91 (s, 3 H), 4.40 (q, 2 H, J = 6.84 Hz), 6.70–7.92 (total 3 H), 10.13 (s, 1 H); IR (Nujol) 3215 (w), 1755 (s), 1670 (s), 1600 (s), 1590 (s), 1520 (s), 1415 (w), 1400–1000 (several strong bands); MS calcd for $C_{17}H_{21}NO_6$ 335.1369, M^+ 335.1384 (17), 290 (19), 289 (100), 274 (19), 203 (28).

Preparation of 6,7-Dimethoxy-3,3-dimethyl-1*H*,3*H*,4*H*,9*H*-furo[3,4-*b*]quinoline-1,9-dione (VI). The chlorobutenolide Vb (3.5 g, 16 mmol) was treated with 3,4-dimethoxyaniline (2.5 g, 16 mmol) and 1.7 g of triethylamine in toluene as described before to give the crude anilinobutenolide Vc, 3.2 g (59%), that was then cyclized by the method used for the preparation of III to give 2.3 g (48% over the two steps) of VI: mp >250 °C; TLC R_f 0.48 (9:1 chloroform–methanol); ^{1}H NMR (DMSO-d_6) δ 1.7 (s, 6 H), 3.93 (s, 3 H), 4.02 (s, 3 H), 7.10 (s, 1 H), 7.62 (s, 1 H); IR (Nujol) 3250–3050 (several weak bands), 1760 (s), 1725 (s), 1630 (s), 1610 (w), 1595 (w), 1575 (w), 1550 (w), 1500 (s), 1400–1000 (several strong bands); MS calcd for $C_{15}H_{15}NO_5$ 289.0949, M^+ 289.0989 (97), 274 (41), 272 (11), 271 (63), 270 (23), 256 (16), 240 (10).

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Registry No. Ia, 106536-15-6; Ib, 106536-17-8; Ic, 106536-16-7; IIa, 125610-66-4; IIb, 125610-67-5; IIc, 125610-68-6; III, 125610-69-7; IVa, 79522-49-9; IVb, 125610-70-0; Va, 55473-58-0; Vb, 125610-71-1; Vc, 125610-73-3; VI, 125610-72-2; $PhNH_2$, 62-53-3; $(CH_3)_2C(Br)COCl$, 20469-89-0; diethyl (ethoxymagnesio)malonate, 35227-78-2; 3,4-dimethoxyaniline, 6315-89-5.

(10) For the preparation of diethyl (ethoxymagnesio)malonate, see, for example: Price, J. A.; Tarbell, D. S. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, p 285.

First Preparation of Mitomycins Specifically Labeled with Deuterium at the C^6-Methyl Position

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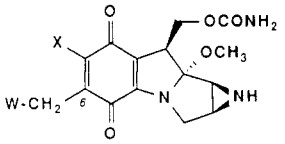
The C^6-methyl group of mitomycins was specifically labeled with deuterium. 7,7-(Ethylenedioxy)mitomycin (8), a masked quinonoid compound derived from mitomycin A (2), played an important role in this methodology.

Mitomycins are well known to be potent antitumor antibiotics,[2] produced by various *Streptomyces* cultures. Among these compounds, mitomycin C (1) has been used extensively in cancer chemotherapy against a variety of

0022-3263/90/1955-2515$02.50/0

solid tumors, but its use is limited by side effects, such as severe bone marrow suppression or gastrointestinal damage. Consequently, a number of derivatives targeting less toxicity or more effective activity have been synthesized in our laboratory[3] or by other groups.[4] For evaluation of these new mitomycin derivatives, drug metabolism and pharmacokinetic studies including a comparison with those of **1** seemed to be very important. In such studies, isotopically labeled drugs such as tritium or carbon-14 have been used in a general way. In the case of **1**, only two methods for labeling were reported to date. One is the Wilzbach method[5] for unilabeling with tritium and another is the biosynthetic method[6] for labeling with carbon-14, but both methods were not practical for selective and effective labeling. Thus the pharmacokinetics of **1** have not been clear, and a practical method for labeling at a metabolically stable position such as C^6-methyl group has long been desired. Herein, we report the first practical method for specific labeling at the C^6-methyl group of mitomycins with deuterium.



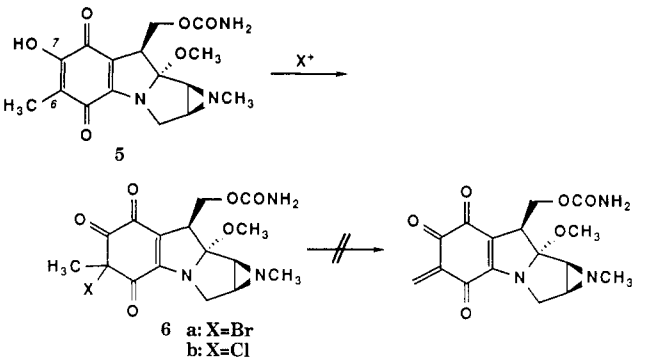
1, Mitomycin C (X=NH_2, W=H)
2, Mitomycin A (X=OCH_3, W=H)
3, X=NH_2, W=D
4, X=OCH_3, W=D

Our initial strategy for introduction of deuterium at C^6-methyl group involved formation of 6-halo 5,7-diketone **6** derived from 7-*O*-demethylmitomycin F (**5**) followed by dehydrohalogenation with a base (Scheme I). Model studies using **6a** and **6b** resulted in the recovery of the starting material **5**. After several attempts failed, we chose to use 7,7-(ethylenedioxy)mitomycin (**8**) as a starting material.

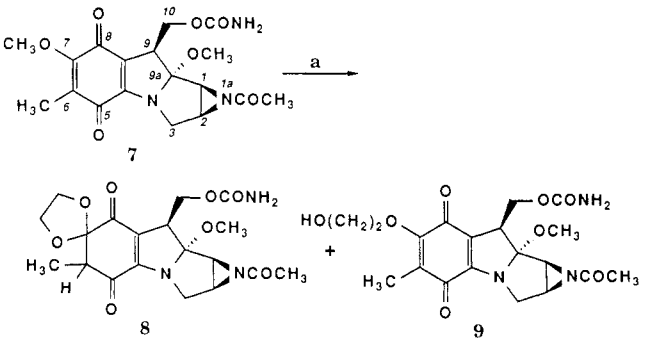
7-Methoxymitomycins have been known to give C^7-transalkoxylation products by a reaction with some alcohols under basic conditions.[7] During the course of our synthesis of new mitomycin derivatives, we found that the reaction of 1a-acetylmitomycin A (**7**) with ethylene glycol in the presence of KOH gave **8** as an inseparable 2.4:1 mixture of isomers at C6, containing an acetal group at C7 and an active methine moiety at C6 in 75% yield, along with 7-(2-hydroxyethoxy)mitosane (**9**) (10% yield)[8] (see Scheme II).

The ethylene acetal **8** was supposed to be a tautomer of **9**, since **8** could be easily converted to mitomycin C (**1**) (NH_3/MeOH) and mitomycin A (**2**) (KOH/MeOH) in 88% and 84% yields, respectively. Introduction of the

Scheme I

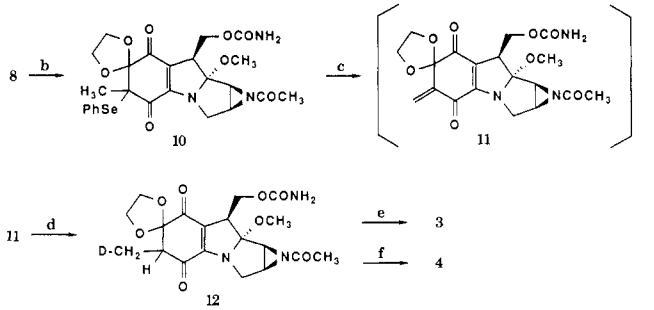


Scheme II[a]



[a] (a) KOH(catalytic), ethylene glycol, THF.

Scheme III[a]



[a] (b) PhSeBr, Et_3N, THF; (c) mCPBA, $CHCl_3$–Py; (d) $NaBD_4$,-CD_3OD; (e) NH_3, MeOH; (f) KOH, MeOH.

phenylselenenyl group at C6 of **8** was accomplished by treatment with PhSeBr in the presence of Et_3N to give 6-phenylselenenyl compound **10** (Scheme III) as a mixture of stereoisomers at the C6 (ca. 4:5 by ^1H NMR spectroscopy) in 91% yield. Oxidative elimination of the phenylselenenyl group (mCPBA/pyridine) of **10** proceeded readily at room temperature. Generation of enone **11** could be directly monitored by ^1H NMR spectroscopy. The structure of the enone **11** was supported by the ^1H NMR spectrum in which the newly generated C^6-methylene group absorbed at δ 6.09 and 6.36 (1 H each) as broad singlets. The conversion yield determined by ^1H NMR analysis was 93% after 10 min. The isolation of **11** was difficult from this reaction mixture, so we used **11** in the next reduction step without any further treatment. $NaBD_4$ was added to this mixture together with CD_3OD to give the 1,4-reduction product **12**. After a usual workup, the crude **12** was treated with NH_3 in MeOH for amination at C7 and deacetylation of the 1a-aziridine nitrogen to give [C^6-*methyl*-$^2H_1$]mitomycin C (**3**) in 26% overall yield from **10**. The specificity of labeling at the C^6-methyl group was confirmed by ^1H, ^2H, and ^{13}C NMR analyses, that is, in

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(2) (a) Carter, S. K.; Crooke, S. T. *Mitomycin C: Current Status and New Developments*; Academic Press: New York, 1978. (b) Remers, W. A. *The Chemistry of Antitumor Antibiotics*; Wiley: New York, 1979.

(3) Kono, M.; Saitoh, Y.; Kasai, M.; Sato, A.; Shirahata, K.; Morimoto, M.; Ashizawa, T. *Chem. Pharm. Bull.* **1989**, *37*, 1128.

(4) Sami, S. M.; Iyenger, B. S.; Tarnow, S. E.; Remers, W. A.; Bradner, W. T.; Schuring, J. E. *J. Med. Chem.* **1984**, *27*, 701 and references cited therein.

(5) (a) Weissbach, A.; Lisio, A. *Biochemistry* **1965**, *4*, 196. (b) Ørstavik, J. *Acta Path. Microbiol. Scand., Sect. B* **1974**, *82*, 270.

(6) Hornemann, U.; Cloyd, J. C. *J. Chem. Soc., Chem. Commun.* **1971**, 301.

(7) Urakawa, C.; Nakano, K.; Imai, T. *J. Antibiot.* **1980**, *33*, 270.

(8) In a similar case, Sami and co-workers[9] have reported that a reaction of mitomycin A (**2**) with ethylene glycol in the presence of KOH gave 7-demethoxy-7-(2-hydroxyethoxy)mitomycin A in 29% yield, but a formation of ethylene acetal at C7 was not mentioned at all. In our experiment, a reaction of **2** with ethylene glycol also gave the corresponding 7,7-ethylenedioxy compound as a major product in 81% yield.

(9) Sami, S. M.; Iyenger, B. S.; Remers, W. A.; Bradner, W. T. *J. Med. Chem.* **1987**, *30*, 168.

the ^{1}H NMR spectrum of **3**, a monodeuteriated C^6-methyl group was observed at δ 1.74 as a triplet (J = 2.2 Hz). The incorporation of deuterium was determined to be 70% by ^{1}H NMR and EIMS analyses. Lower incorporation of deuterium seemed to result from some proton sources in this reaction mixture. In addition, the labeled mitomycin A (**4**), which has been useful for the synthesis of C^7-substituted mitomycins, was obtained by treatment of **12** with catalytic KOH in methanol in 31% overall yield from **10**.

In this way, we have developed a practical method for the synthesis of C^6-methyl-labeled mitomycins. This methodology is applicable to the synthesis of tritium labeled mitomycins, and studies of pharmacokinetics and mechanism of action of mitomycins will be progressively extended by using the radioactive drug. Furthermore, this methodology provides a new approach to the synthesis of hitherto inaccessible mitomycin derivatives directed toward more effective and less toxic antitumor agents.

Experimental Section

Unless otherwise noted, materials were obtained from commercial suppliers and were used without purification. Nuclear magnetic resonance spectra were determined on a Bruker AM 400 instrument. Mass spectra were determined on a Hitachi M-80B mass spectrometer. Infrared spectra were determined on a Nihon Bunko IR-810 instrument. Ultraviolet–visible spectra were determined on a Hitachi 228 spectrophotometer. High-performance liquid chromatography was performed on a JASCO Trirotar II equipped with a Shimadzu SPD M6A photodiode-array ultraviolet–visible detector.

1a-Acetyl-7-demethoxy-6,7-dihydro-7,7-(ethylenedioxy)-mitomycin A (8). To a solution of 1a-acetylmitomycin A (**7**) (148 mg, 0.379 mmol) in 3.5 mL of tetrahydrofuran and 2 mL of ethylene glycol at 25 °C was added 0.5 mL of an ethylene glycol solution containing 1.6% (w/w) of potassium hydroxide. The solution was stirred at 25 °C for 5 h. To the reaction mixture was added an excess of dry ice. The solution was diluted with chloroform and washed with brine. The organic layer was dried and concentrated. The crude product was purified by silica gel chromatography using 3% methanol in chloroform to afford 120 mg (75% yield) of **8** as a 2.4:1 mixture of two diastereomers at C6 and 16 mg (10%) of **9**. Compound **9** was easily converted into **8** in a solution of $CDCl_3$ and afforded a 4.5:1 mixture of **8** and **9** after 10 min.

8: 400-MHz ^{1}H NMR ($CDCl_3$) (major) δ 1.20 (3 H, d, J = 6.6 Hz, 6-CH_3), 2.11 (3 H, s, 1a-$COCH_3$), 3.21 (3 H, s, 9a-OCH_3), 3.22 (1 H, q, J = 6.6 Hz, 6-H), 3.23 (1 H, dd, J = 4.4, 2.0 Hz, 2-H), 3.47 (1 H, dd, J = 13.1, 2.0 Hz, 3α-H), 3.50 (1 H, d, J = 4.4 Hz, 1-H), 3.73 (1 H, dd, J = 10.8, 4.9 Hz, 9-H), 4.04 (1 H, d, J = 13.1 Hz, 3β-H), 3.98–4.41 (4 H, m, OCH_2CH_2O), 4.17 (1 H, t, J = 11.1 Hz, 10-Ha), 4.82 (2 H, br s, $OCONH_2$), 4.98 (1 H, dd, J = 11.1, 4.9 Hz, 10-Hb); (minor (main peaks)) δ 1.24 (3 H, d, J = 6.9 Hz, 6-CH_3), 2.11 (3 H, s, 1a-$COCH_3$), 3.04 (1 H, q, J = 6.9 Hz, 6-H), 3.22 (3 H, s, 9a-OCH_3), 3.42 (1 H, dd, J = 13.0, 1.7 Hz, 3α-H), 4.34 (1 H, d, J = 13.0 Hz, 3β-H), 4.89 (1 H, dd, J = 10.8, 4.9 Hz, 10-Hb); SIMS m/z 422 (M^+ + 1); IR (KBr) 3292, 2900, 1720, 1700, 1645, 1575, 1448, 1328, 1268, 1189, 1067, 1031 cm^{-1}; UV (CH_3CN) 237 (log ϵ 4.07), 359 (3.97) nm. Anal. Calcd for $C_{19}H_{23}N_3O_8$: C, 54.15; H, 5.50; N, 9.97. Found: C, 54.26; H, 5.72; N, 9.70.

9 (a 2.6:1.9:1 mixture of **8** major, **8** minor, and **9**): 400-MHz ^{1}H NMR ($CDCl_3$) δ 1.92 (3 H, s, 6-CH_3), 3.51 (1 H, dd, J = 13.3, 2.2 Hz, 3α-H), 3.71 (1 H, dd, J = 10.8, 4.8 Hz, 9-H), 3.86 (2 H, br q, J = 4.6 Hz, 7-OCH_2CH_2OH), 4.30 (2 H, m, 7-OCH_2CH_2OH); SIMS m/z 424 (M^+ + 3), 423 (M^+ + 2), 422 (M^+ + 1). HPLC (YMC AM 312 ODS S-5 μm; 150 × 6 mm i.d.; 50:50 CH_3CN–H_2O at 1.0 mL/min): **8**, retention time 3.93 min; UV 241, 365 nm; **9**, retention time 3.40 min; UV–visible 216, 326, 520 nm.

1a-Acetyl-7-demethoxy-6,7-dihydro-7,7-(ethylenedioxy)-6-(phenylselenenyl)mitomycin A (10). To a solution of ethylene acetal **8** (120 mg, 0.285 mmol) in 4 mL of tetrahydrofuran and 0.3 mL of triethylamine at 25 °C was added 127 mg of phenylselenenyl bromide. The reaction was stirred at 25 °C for 50 min, and then the solvent was removed in vacuo. The crude product was purified by silica gel chromatography using 3% methanol in chloroform to afford 149 mg (91% yield) of **10** as a 2:1 mixture of two diastereomers at C6.

10: 400-MHz ^{1}H NMR ($CDCl_3$/py-d_5, 8/1) (major) δ 1.44 (3 H, s, 6-CH_3), 2.18 (3 H, s, 1a-$COCH_3$), 3.16 (3 H, s, 9a-OCH_3), 3.27 (1 H, dd, J = 4.5, 2.0 Hz, 2-H), 3.39 (1 H, dd, J = 13.0, 2.0 Hz, 3α-H), 3.51 (1 H, d, J = 4.5 Hz, 1-H), 3.80 (1 H, dd, J = 11.1, 4.8 Hz, 9-H), 3.84 (1 H, d, J = 13.0 Hz, 3β-H), 3.95–4.50 (4 H, m, OCH_2CH_2O), 4.28 (1 H, t, J = 11.1 Hz, 10-Ha), 5.15 (1 H, dd, J = 11.1, 4.8 Hz, 10-Hb), 5.80 (2 H, br s, $OCONH_2$), 7.24–7.63 (5 H, m, 6-SeC_6H_5); (minor (main peaks)) δ 1.42 (3 H, s, 6-CH_3), 2.18 (3 H, s, 1a-$COCH_3$), 3.22 (1 H, dd, J = 4.4, 2.0 Hz, 2-H), 3.26 (3 H, s, 9a-OCH_3), 3.38 (1 H, dd, J = 13.0, 2.0 Hz, 3α-H), 3.47 (1 H, d, J = 4.4 Hz, 1-H), 3.75 (1 H, dd, J = 11.0, 4.7 Hz, 9-H), 4.14 (1 H, t, J = 10.8 Hz, 10-Ha), 4.54 (1 H, d, J = 13.0 Hz, 3β-H), 4.85 (1 H, dd, J = 11.0, 4.7 Hz, 10-Hb); SIMS m/z 577/575 (M^+ + 1); IR (KBr) 3460, 1725, 1688, 1656, 1571, 1435, 1377, 1334, 1247, 1205, 1066, 1031 cm^{-1}; UV (CH_3CN) 218 sh (log ϵ 4.20), 254 (4.06), 359 (3.73) nm. Anal. Calcd for $C_{25}H_{27}N_3O_8Se$: C, 52.09; H, 4.72; N, 7.29. Found: C, 51.99; H, 4.78; N, 7.23.

[*C*^{6}-*methyl*-^{2}H$_1$]Mitomycin C (3). To a magnetically stirred solution of phenylselenenyl compound **10** (201 mg, 0.349 mmol) in 3.5 mL of chloroform and 0.5 mL of pyridine at 0 °C was added 129 mg (70% purity) of *m*-chloroperbenzoic acid. The reaction was stirred at 0 °C for 25 min. The generation of the enone **11** was confirmed by the reaction in an NMR tube in a similar manner to that described above. To a solution of **10** (6.8 mg, 0.0118 mmol) in 0.4 mL of chloroform-*d* and 0.05 mL of pyridine-d_5 in an NMR tube at 25 °C was added 3.5 mg of *m*-chloroperbenzoic acid. After 10 min at 25 °C, the signals of the C^6-methyl groups (δ 1.42 and 1.44 ppm) of **10** completely disappeared and the characteristic signals of the C^6-methylene group of the generated **11** were observed at δ 6.09 and 6.36 ppm as broad singlets. SIMS of **11** was measured directly using the reaction mixture.

11: 400-MHz ^{1}H NMR ($CDCl_3$/py-d_5, 8/1) δ 2.08 (3 H, s, 1a-$COCH_3$), 3.17 (3 H, s, 9a-OCH_3), 3.24 (1 H, dd, J = 4.4, 2.0 Hz, 2-H), 3.49 (1 H, dd, J = 13.3, 2.0 Hz, 3α-H), 3.50 (1 H, d, J = 4.4 Hz, 1-H), 3.79 (1 H, dd, J = 11.1, 4.8 Hz, 9-H), 4.17 (1 H, t, J = 10.8 Hz, 10-Ha), 4.04–4.29 (4 H, m, OCH_2CH_2O), 4.38 (1 H, d, J = 13.3 Hz, 3β-H), 5.04 (1 H, dd, J = 10.8, 4.8 Hz, 10-Hb), 5.79 (2 H, br s, $OCONH_2$), 6.09 (1 H, br s, C_6=CH_{2a}), 6.36 (1 H, br s, C_6=CH_{2b}); SIMS m/z 420 (M^+ + 1), $C_{19}H_{21}N_3O_8$ = 419.

To the resultant solution was added 7.3 mg of sodium borodeuteride in 0.2 mL of methanol-d_4. The solution was stirred at 0 °C for 40 min and diluted with 0.067 M phosphate buffer (pH 4.0) and chloroform. The organic layer was washed with sodium bicarbonate and brine and then dried. After removal of the solvent in vacuo, the crude product was purified by silica gel chromatography using 3% methanol in chloroform to afford **12**. The production of **12** was confirmed by the observed R_f 0.52, by silica gel TLC using a solvent system of 10% methanol in chloroform, identical with that of **8**. To a solution of **12** in 0.2 mL of methanol at 25 °C was added 0.5 mL of 6.8 M ammonia/methanol. The solution was stirred at 25 °C for 5 h, and then the solvent was removed in vacuo. The crude product was purified by silica gel chromatography using 10% methanol in chloroform. Recrystallization from chloroform afforded 30 mg (26% yield from **10**) of **3**. The incorporation of deuterium was determined to be 70% on the basis of ^{1}H NMR and EIMS analyses.

3: 400-MHz ^{1}H NMR ($CDCl_3$) δ 0.9 (1 H, br s, 1a-NH), 1.74 (1.4 H, t, J = 2.2 Hz, 6-CH_2D), 1.76* (0.9 H, s, 6-CH_3), 2.82 (1 H, dd, J = 4.4, 1.7 Hz, 2-H), 2.89 (1 H, d, J = 4.4 Hz, 1-H), 3.21 (3 H, s, 9a-OCH_3), 3.51 (1 H, dd, J = 12.8, 1.7 Hz, 3α-H), 3.63 (1 H, dd, J = 10.6, 4.4 Hz, 9-H), 4.25 (1 H, d, J = 12.8 Hz, 3β-H), 4.53 (1 H, br t, J = 10.6 Hz, 10-Ha), 4.71 (1 H, dd, J = 10.6, 4.4 Hz, 10-Hb), 4.71 (2 H, br s, $OCONH_2$), 5.20 (2 H, br s, 7-NH_2); 61-MHz ^{2}H NMR (DMSO) δ 1.61 (br s); 100-MHz ^{13}C NMR (py-d_5) δ 8.50 (t, J = 19.6 Hz, 6-CH_2D), 8.76* (6-CH_3), 32.61 (2), 36.74 (1), 44.29 (9), 49.57 (9a-OCH_3), 50.63 (3), 62.55 (10), 104.30 (6-*C*-CH_2D), 104.34* (6-*C*-CH_3), 106.80 (9a), 110.84 (8a), 149.51 (7), 156.08 (4a), 158.09 ($OCONH_2$), 176.75 (8), 178.45 (5) (*, peaks due to natural mitomycin C (**1**)); EIMS m/z 335 (M^+); HRMS m/z for $C_{15}H_{17}DN_4O_5$ calcd 335.1340, found 335.1366; IR (KBr) 3450, 3320, 1709, 1592, 1543, 1442, 1339, 1219, 1061 cm^{-1}.

[*C*^{6}-*methyl*-^{2}H$_1$]Mitomycin A (4). In a similar manner to that described above, compound **12** was prepared. To a me-

chanically stirred solution of **12** (70 mg, 0.166 mmol) in 3 mL of methanol at 20 °C was added 1.0 mg of potassium hydroxide. The solution was stirred at 20 °C for 12 h. To the reaction mixture was added small pieces of dry ice. The solution was diluted with chloroform and washed with brine. The organic layer was dried and concentrated. The crude product was purified by silica gel chromatography using 5% methanol in chloroform to afford 37 mg (64% yield from **12**) of **4**.

4: 400-MHz ^{1}H NMR ($CDCl_3$) δ 0.7 (1 H, br s, 1a-NH), 1.82 (1.4 H, t, J = 2.2 Hz, 6-CH_2D), 1.85* (0.9 H, s, 6-CH_3), 2.83 (1 H, dd, J = 4.2, 1.7 Hz, 2-H), 2.92 (1 H, d, J = 4.2 Hz, 1-H), 3.22 (3 H, s, 9a-OCH_3), 3.48 (1 H, dd, J = 12.8, 1.7 Hz, 3α-H), 3.62 (1 H, dd, J = 10.6, 4.4 Hz, 9-H), 4.04 (3 H, s, 7-OCH_3), 4.05 (1 H, d, J = 12.8 Hz, 3β-H), 4.56 (1 H, t, J = 10.6 Hz, 10-Ha), 4.74 (1 H, dd, J = 10.6, 4.4 Hz, 10-Hb), 4.89 (2 H, br s, $OCONH_2$) (*, peak due to natural mitomycin A (**2**)); 61-MHz ^{2}H NMR ($CHCl_3$) δ 1.85 (t, J = 2.2 Hz); EIMS m/z 350 (M^+); HRMS m/z for $C_{16}H_{18}DN_3O_6$ calcd 350.1337, found 350.1347; IR (KBr) 3380, 1706, 1633, 1551, 1434, 1323, 1219, 1061 cm^{-1}.

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Registry No. **3**, 125685-49-6; **4**, 125685-50-9; **7**, 4901-84-2; **8** (isomer 1), 125761-41-3; **8** (isomer 2), 125761-42-4; **9**, 125685-48-5; **10** (isomer 1), 122675-60-9; **10** (isomer 2), 122644-74-0; **11**, 122644-75-1; **12**, 122644-76-2.

Reductive Transformations of 10-Deoxydaunomycinone[†,1,2]

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An enzyme system consisting of spinach ferredoxin-$NADP^+$ reductase (ferredoxin reductase; EC 1.18.1.2) and pig heart isocitric dehydrogenase($NADP^+$) (isocitric dehydrogenase; EC 1.1.1.42), along with spinach ferredoxin and either of the coenzymes NAD(P)H, was utilized in the reduction of aqueous anaerobic solutions of daunomycin and related aglycons. Typically, reductive transformations of daunomycin yielded three major and several minor aglycon products, as detected by reverse-phase liquid chromatography analysis. The ferredoxin reductase component of the enzymatic system elicited the reductive deglycosylation of the daunomycin. Subsequent to the loss of the daunosamine sugar were several keto-enol tautomeric equilibria of the hydroquinone intermediates that result in the transformation of the anthracycline ring system in a fashion identical with that observed during the sodium dithionite reduction of daunomycin (Brand, D. J.; Fisher, J. *J. Am. Chem. Soc.* **1986**, *108*, 3088-3096). Additionally, an aldo-keto reductase contaminant in the isocitric dehydrogenase enzyme preparation caused the stereoselective reduction of the acetyl side chain found in daunomycin and related aglycons, resulting in all the product aglycons having a 1-hydroxyethyl side chain bearing an *S* configuration at the C-1′ stereogenic center. The three major products of the ferredoxin reductase and isocitric dehydrogenase enzyme-catalyzed reduction of daunomycin have been characterized. Two of the major products of the reaction, [*S*-(*R**,*S**)]-1,2,3,4-tetrahydro-2,11-dihydroxy-2-(1-hydroxyethyl)-7-methoxy-5,12-naphthacenedione (**16**; 50–55% of the product) and [*S*-(*R**,*S**)]-1,2,3,4-tetrahydro-2,6-dihydroxy-2-(1-hydroxyethyl)-7-methoxy-5,12-naphthacenedione (**17**; 15–20% of the product), involve both the loss of an oxygen from the anthracycline's C ring and the reduction of the side-chain acetyl group. The third major product and two of the minor products were a diastereomeric set of "leuco" tautomers of the hydroquinone produced upon reduction of the anthracycline ring system; reduction of the acetyl side chain also occurred. These three diastereomers of [2*R*-[2α,2(*S**)]]-1,2,3,4,4a,12a-hexahydro-2,6,11-trihydroxy-2-(1-hydroxyethyl)-7-methoxy-5,12-naphthacenedione differ in the stereochemistry at the C-4a, C-12a ring juncture. The major diastereomer (**19**; 20–25% of the product) has a trans ring juncture, while the two minor diastereomers (**20** and **21**; 0–3% and 5–8% of the product) have a cis ring juncture. Virtually identical results were observed when either daunomycinone or (1′*S*)-1′-dihydrodaunomycinone (**11**), instead of daunomycin, were used as a substrate for the enzyme system. However, a chromatographically different set of products, epimeric to those formed when (1′*S*)-1′-dihydrodaunomycinone was the substrate, were produced when (1′*R*)-1′-dihydrodaunomycinone (**12**) was used. All of these products had the *R* configuration at the C-1′ stereogenic center. This series of results may prove of value in the synthetic preparation of 1′-anthracyclinols and in the study of the possible role of 1′-anthracyclinol aglycons and glycosides in the expression of the anthracycline's dose-limiting cardiotoxicity.

A viable antineoplastic agent is one that is selectively toxic to the neoplasm rather than toward the host organism. Quinones account for a disproportionate number of these cytotoxic substances. This functional group is found in two of the most useful antitumor antibiotics classes (mitomycins and anthracyclines), as well as in several of the newer antitumor structures (e.g., mitoxantrone and fredericamycin). The cytotoxicity of the quinones has led to the speculation that there is an intrinsic chemical property of the quinone moiety that expresses antitumor activity.[3] An obvious possibility is the quinone's capacity

[†] This paper is dedicated to the memory of Paul F. Wiley, in recognition of his achievements in natural product chemistry, in general, and in the anthracyclines in particular.

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(1) For indexing purposes the anthracyclinone ring system is numbered in this paper in accordance with the numbering system of *Chemical Abstracts*, which is at variance with the numbering system introduced by Brockmann[2] that is used by most workers in the anthracycline field. However, for reasons of convenience to the reader, the 8-(1-hydroxyethyl) aglycons will be referred to as 1′-dihydro aglycons.

(2) Brockmann, H. *Prog. Chem. Org. Nat. Prod.* **1963**, *21*, 121-182.

(3) For recent, general discussions of this phenomenon, see: (a) Pullman, B. *Int. J. Quantum Chem., Quantum Biol. Symp.* **1986**, *13*, 95-105. (b) Cohen, G. M.; d'Arcy Doherty, M. *Br. J. Cancer, Suppl.* **1987**, *55*, 46-52. (c) Butler, J.; Hoey, B. M. *Br. J. Cancer, Suppl.* **1987**, *55*, 53-59.

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