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Note

7-*O*-(3-Amino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl)daunomycinone, a configurational analog of daunorubicin

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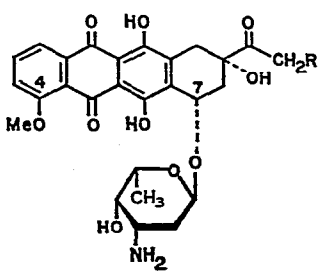
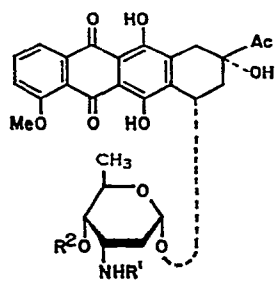
The anthracycline glycosides daunorubicin (**1**) and adriamycin (**2**) exhibit outstanding therapeutic efficacy against a broad spectrum of human tumors[1]. Their administration is, however, accompanied by various undesirable side-effects, especially a cumulative, dose-related cardiopathogenicity[2], which seriously impedes their broader utilization in chemotherapy. The hypothesis that small, structural alterations of the parent drugs may favorably modify the therapeutic properties (compare the minor chemical difference between **1** and **2** and the pronounced difference in their antitumor activities) has motivated an intensive search (see, for example, refs. 3–7) for new derivatives and analogs of **1** and **2** that might display improved therapeutic



1 R = H
2 R = OH



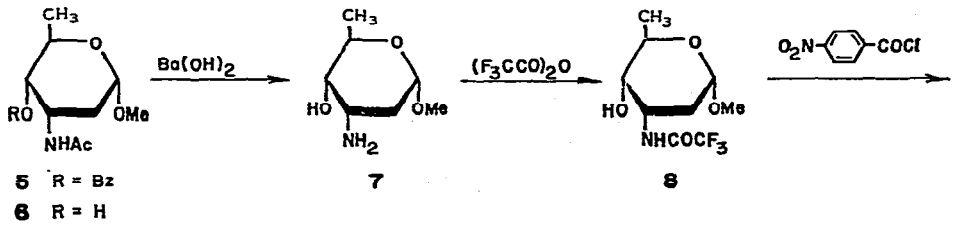
3 $R^1 = F_3CCO$; $R^2 = p\text{-}O_2NC_6H_4CO$
4 $R^1 = R^2 = H$

indices, namely, greater effectiveness or decreased toxicity, or both. The derivative AD32 (3′-*N*-trifluoroacetyladriamycin 14-pentanoate[6,8]), for example, and the 4-demethoxy[4,6,9] (aglycon-moiety modified) and 3′-deamino-3′-hydroxy[10] (sugar-portion altered) analogs of **1** and **2** constitute a selection of the more promising candidates that have recently been introduced and which are undergoing extensive biological testing. Continuing our program[7,10] directed towards the synthesis of anthracycline glycosides modified in the daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose) residue, we now describe the preparation and early biological evaluation

of a configurational analog (**4**) of **1** in which 3-amino-2,3,6-trideoxy-D-*ribo*-hexose (D-ristosamine) constitutes the carbohydrate component.

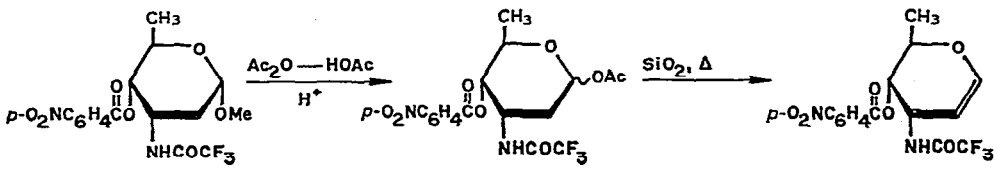
The point of departure was methyl 3-acetamido-4-*O*-benzoyl-2,3,6-trideoxy-α-D-*ribo*-hexopyranoside[11] (**5**), a key intermediate in the daunosamine synthesis[12]; it is prepared most conveniently, in seven steps, from methyl α-D-mannopyranoside. This route[12] involves, as major features, direct generation of a 2-deoxy-3-keto intermediate whose oxime is reduced almost stereospecifically, to introduce the correctly oriented 3-amino group. In contrast, most other procedures[13–18] advocated for the preparation of (D or L) ristosamine derivatives employ a 1,2-unsaturated ("D-glucal" or "L-rhamnal") intermediate to effect deoxygenation at C-2, and the 3-amino group is generally introduced by way of a sulfonate[13–16,19] or allylic[18] azide-displacement–hydrogenation sequence.

Treatment of the peracylated methyl glycoside[11] **5**, or its debenzoylated analog[11] **6** with hot, aqueous barium hydroxide afforded, in excellent yield, crystalline methyl 3-amino-2,3,6-trideoxy-α-D-*ribo*-hexopyranoside (**7**), whose L enantiomorph has already been reported by Acton *et al.*[15] and, independently, by Bognar *et al.*[16].



5 R = Bz
6 R = H

7 **8**



9 **10** **11**

Trifluoroacetylation of **7**, followed by removal of the 4-*O*-trifluoroacetyl group by methanolysis, furnished the partially protected glycoside **8** as a (distillable) syrup. Subsequent, conventional *p*-nitrobenzoylation of **8** (whose L enantiomorph has recently been described by two independent laboratories[16,17]) gave the crystalline 4-*p*-nitrobenzoate **9** in high (87%) yield. Although thorough examination of detailed, characterizing data (elemental analysis, i.r. spectrum, ^{1}H-n.m.r. spectroscopy, and mass spectrometry; see Experimental section) left no doubt about the identity and homogeneity of **9**, its physical constants (m.p. 78–80°, $[\alpha]_D$ +94° in chloroform) showed significant deviation from values (m.p. 174–176°, $[\alpha]_D$ −124.2° in chloroform) reported[17] for the L enantiomorph of **9**, and the reason for this situation is not

clear. Furthermore, acid hydrolysis of **9**, according to a literature precedent[17] for the L enantiomorph, did not lead in our hands to a unique product (the reducing sugar analog of **9**), but rather to a mixture of products (presumably partially and fully deprotected sugars). Therefore, the methyl glycoside **9** was subjected to acetolysis, which effectively cleaved the aglycon without concurrent loss of protecting groups, and afforded the 1-acetate **10** as a 2:1 mixture of the α and β anomers. Without attempting separation of this mixture, the acetolysis product **10** was treated, by the elimination procedure recently described[7], at elevated temperature (110°) with mild acid (silica gel), to yield the glycal **11** as a distillable syrup in acceptable (65%) yield.

Treatment of this glycal (**11**) with daunomycinone in benzene in the presence of a catalytic amount of *p*-toluenesulfonic acid gave, after chromatographic purification, the protected anthracycline glycoside **3** as the only product isolated in substantial yield, although t.l.c. analysis of the reaction product had revealed the presence of a faster-migrating product, visible as a red zone, but insufficient for isolation. The α-D-anomeric configuration of the glycosidic linkage in **3** was readily determined by ^{1}H-n.m.r. spectroscopy from the pattern (narrow multiplet, splittings of ~3 and 1 Hz) of the H-1′ signal, which fell at δ 5.62 (in acetone-d_6).

Removal of the protecting groups in **3** was accomplished with dilute aqueous sodium hydroxide, to afford the target compound **4**, isolated as its amorphous hydrochloride salt.

The antitumor activity of the new daunomycinone glycoside **4** was assayed *in vivo* against P388 lymphocytic leukemia in CDF_1 mice. Preliminary results are listed in Table I and are compared with data obtained concurrently for daunorubicin (**1**) and adriamycin (**2**). In this test system, compound **4** showed antitumor activity

TABLE I

ACTIVITY[a] OF DAUNORUBICIN (**1**), ADRIAMYCIN (**2**), AND THE 1′,5′-*diepi*-DAUNORUBICIN (**4**) ON P388 LYMPHOCYTIC LEUKEMIA IN MICE[b]

Compound **1** *(NSC 82151)*		*Compound* **2** *(NSC 123127)*		*Compound* **4** *(NSC 309694)*	
Dose[c] *(mg/kg)*	*T/C*[d] *(%)*	*Dose*[c] *(mg/kg)*	*T/C*[d] *(%)*	*Dose*[c] *(mg/kg)*	*T/C*[d,e] *(%)*
16	114	16	168	50	125
8	107	8	150	25	110
4	115	4	145	12.5	120
2	127	2	120	6.25	124
1	120	1	120	3.13	111

[a]Data obtained under the auspices of the National Cancer Institute, Division of Cancer Treatment, Drug Research and Development Branch. [b]CDF_1 mice were injected i.p. with 10^6 P388 lymphocytic leukemia cells on day 0, and treated i.p. on days 5, 9, and 13 with the drug dose specified. [c]Six mice per dose level. [d]Ratio of median survival-time expressed as percent of untreated controls; T/C values >125 indicate presumptive activity. No significant, acute, toxic drug deaths (survival less than 5 days after initiation of treatment) were seen at any of the dose levels reported. [e]The protected derivative **3** (NSC 305989) showed T/C 111 under the same test-conditions at a dose level of 25 mg/kg.

comparable with that of daunorubicin (**1**), but substantially higher dose-levels were required than when **1** or (the distinctively more-effective) adriamycin (**2**) were used. The low, albeit appreciable, antitumor activity of **4** (which differs from the parent drug **1** in the stereochemistry at C-1′ and C-5′) could not have been anticipated in advance, in particular when compared with the corresponding 3′-deamino-3′-hydroxy analogs[10] of **1** and **4** which, respectively, display high (α-L-*lyxo* configuration) and negligible (α-D-*ribo*) antitumor activity. However, these biological data are preliminary, and more information will be needed in order to assess the pharmacological potential (if any) of anthracycline glycosides of the D series.

EXPERIMENTAL

General methods. — Solvents were evaporated under diminished pressure at bath temperatures below 50°. T.l.c. was performed on precoated plates of Silica Gel 60 (E. Merck, Darmstadt); zones were detected by u.v. light, and by spraying with sulfuric acid and subsequent heating. Melting points were determined with a Thomas–Hoover apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. I.r. spectra were recorded with a Perkin–Elmer Model 457 grating i.r. spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 100 MHz with a Varian HA-100 spectrometer; chemical shifts refer to an internal standard of tetramethylsilane (δ = 0.00). Mass spectra were recorded with an AEI MS-9 double-focusing, high-resolution spectrometer (ionizing and accelerating potentials, 70 eV and 8 kV). Microanalyses were performed by W. N. Rond. X-Ray powder diffraction data give interplanar spacings, Å, for CuKα radiation. The camera diameter was 114.59 mm. Relative intensities were estimated visually: m, moderate; s, strong; v, very; w, weak. The strongest lines are numbered (1, strongest); double numbers indicate approximately equal intensities.

*Methyl 3-amino-2,3,6-trideoxy-*α-D-ribo-*hexopyranoside* (**7**). — A mixture of methyl 3-acetamido-4-*O*-benzoyl-2,3,6-trideoxy-α-D-*ribo*-hexopyranoside[11] (**5**; 7.15 g, 23.26 mmol) and barium hydroxide octahydrate (25 g, 79.25 mmol) in water (250 mL) was stirred magnetically while boiling under reflux. After 30 min, all of the starting glycoside **5** had dissolved, and, after 8 h, t.l.c. (2:3 benzene–acetone) revealed that saponification was complete. Solid carbon dioxide was added, the inorganic material was filtered off, and the filtrate was treated with Amberlite IRA-400 (HO^-) resin (80 mL). The strongly alkaline solution was then concentrated to ~100 mL, and lyophilized. The residue (4.11 g), still containing some barium carbonate, was taken up in chloroform (70 mL), the suspension decolorized (activated charcoal) and filtered, and the filtrate evaporated, to give crude **7** (3.58 g, 95%) that was recrystallized from ether–hexane; m.p. 81–83°, $[\alpha]_D^{22}$ +219° (*c* 2, chloroform); ^{1}H-n.m.r. (chloroform-*d*): δ 4.70 (1 H, narrow m, $J_{1,2}$ ~0 Hz, $J_{1,2'}$ 1 Hz, H-1), 3.62 (1 H, dq, $J_{4,5}$ 9 Hz, $J_{5,6}$ 6 Hz, H-5), 3.40–3.30 (2 H, m, H-3,4), 3.32 (3 H, s, OMe), 2.51 (3 H, br s, OH, NH_2), 2.10–1.90 (2 H, m, H-2,2′), and 1.30 (3 H, d, H-6); *m/e* (rel. intensity): 162 (<1, M + 1), 143 (15, M − H_2O), 130 (21, M − MeOH), 117 (7, M − MeCHO),

104 (31), 100 (18), 86 (60, H_2N-CH=CH-CH=OMe^+), 72 (93), and 58 (100, H_2C=CH-OMe^+); X-ray powder diffraction data: 7.37 s (3), 6.80 s (2), 5.61 m, 4.88 m, 4.46 vs (1), 4.23 m, 3.94 m, 3.69 vw, 3.32 m, and 3.23 w.

Anal. Calc. for $C_7H_{15}NO_3$ (161.20): C, 52.16; H, 9.38; N, 8.69. Found: C, 52.21; H, 9.29; N, 8.46.

Alternatively, compound **7** could be prepared from the partially protected methyl 3-acetamido-2,3,6-trideoxy-α-D-*ribo*-hexopyranoside[11] (**6**; 1.22 g, 6.0 mmol) by the procedure just described in the preceding experiment, to afford 850 mg (88%) of the title compound.

The L enantiomorph of **7** was reported[15] to have m.p. 74–76°, $[\alpha]_D$ −213° in chloroform, and[16] to be a hygroscopic foam having $[\alpha]_D$ −192°.

*Methyl 2,3,6-trideoxy-3-trifluoroacetamido-α-*D-ribo-*hexopyranoside* (**8**). — The unprotected glycoside **7** (2.44 g, 15.14 mmol) was dissolved in dry ether (50 mL), and trifluoroacetic anhydride (14 mL, 98.7 mmol) was added with cooling. The clear solution was kept for 15 min at 0° and 3 h at 25°, and then evaporated to a syrup that was dissolved in dry methanol (100 mL) to remove the 4-*O*-trifluoroacetyl group. After 18 h at 25°, the solvent was evaporated off, to afford **8** as a chromatographically homogeneous syrup; yield 3.56 g (91%). To secure analytical data, a sample was distilled at 110° (bath temp.)/100 mtorr; $[\alpha]_D^{26}$ +44.5° (*c* 3.3, chloroform); $\nu_{max}^{CHCl_3}$ 3380 (OH, NH), 1720 and 1545 cm^{-1} (amide); ^{1}H-n.m.r. (chloroform-*d*): δ 8.04 (1 H, br d, $J_{3,NH}$ 7 Hz, NH-3), 4.76 (1 H, narrow m, $J_{1,2} = J_{1,2'} = \sim 1$ Hz, H-1), 4.44 (1 H, m_6, $J_{3,4}$ 4 Hz, H-3), 3.66 (1 H, dq, $J_{4,5}$ 9.8 Hz, $J_{5,6}$ 6 Hz, H-5), 3.50 (1 H, dd, H-4), 3.38 (3 H, s, OMe), 3.17 (1 H, br s, OH), 2.10–1.90 (2 H, complex m, H-2,2'), and 1.26 (3 H, d, H-6). The mass spectrum was readily classified into the characteristic families of fragments proposed[20] for related glycosides: *m/e* (rel. intensity): 257 (M^+, absent) 239 (0.8, B_1), 226 (7, C_1), 225 (4, A_1), 213 (3, D_1) 207 (18, A_2), 199 (6, E_1^2), 192 (3, A_3), 183 (17, F_1^1), 82 (3, F_1^2), 168 (8, F_2^1), 155 (65, D_2), 142 (1, B_2), 140 (17, H_1), 113 (20, C_2), 95 (4, C_3), 69 (46, CF_3^+), 59 (*O*-methyloxiranyl cation), and 58 (65, E_1^1).

Anal. Calc. for $C_9H_{14}F_3NO_4$ (257.21): C, 42.03; H, 5.49; N, 5.45. Found: C, 42.15; H, 5.73; N, 5.38.

For the L enantiomorph of **8**, the following specific rotations have been reported: $[\alpha]_D$ −64° in methanol[16] and −61.9° in benzene[17].

*Methyl 2,3,6-trideoxy-4-*O-p-*nitrobenzoyl-3-trifluoroacetamido-α-*D-ribo-*hexopyranoside* (**9**). — *p*-Nitrobenzoyl chloride (3.8 g, 20.5 mmol) was added to a cold (0°) solution of the partially protected glycoside **8** (3.0 g, 11.66 mmol) in pyridine (30 mL). Conventional processing after 16 h at 25° afforded crude **9**, which was recrystallized from hexane; yield 4.14 g (87%); m.p. 78–80°, $[\alpha]_D^{22}$ +94° (*c* 1, chloroform); ν_{max}^{KBr} 3400 (NH), 1725 (ester C=O, Amide I), and 1530 cm^{-1} (Amide II, NO_2); ^{1}H-n.m.r. (chloroform-*d*): δ 8.35–7.95 (5 H, m, aryl-H. NH), 4.94 (1 H, dd, $J_{3,4}$ 3.9 Hz, $J_{4,5}$ 10 Hz, H-4), 4.84 (1 H, narrow m, $J_{1,2}$ 3.8, $J_{1,2'}$ 1.3 Hz, H-1), 4.73 (1 H, m_6, $J_{2,3}$ 3.8, $J_{2',3}$ 2.9 Hz, H-3), 4.13 (1 H, dq, $J_{5,6}$ 6 Hz, H-5), 3.46 (3 H, s, OMe), 2.23 (1 H, m_6, $J_{2,2'}$ 14.7 Hz, H-2), 1.99 (1 H, ddd, H-2'), and 1.27 (3 H,

d, H-6); *m/e* (rel. intensity): 406 (0.2, M^+), 375 (2, M — MeO·), 362 (1.3, M — MeCHO), 239 (4.3, M — $O_2NC_6H_4CO_2H$), 207 (14, 239 — MeOH), 195 (9, 362 — $O_2NC_6H_4CO_2H$), 150 (100, $O_2NC_6H_4CO^+$), 120 (24), 104 (16), 95 (11, methylpyrylium cation), 69 (7, CF_3^+), and 58 (37, $H_2C{=}CH\text{-}OMe^+$); X-ray powder diffraction data: 8.75 w, 8.11 w, 6.06 w, 5.73 s (1), 5.34 m (3), 5.06 w, 4.69 w, 4.32 m (2), and 3.87 w.

Anal. Calc. for $C_{16}H_{17}F_3N_2O_7$ (406.32): C, 47.30; H, 4.22; N, 6.89. Found: C, 47.12; H, 4.30; N, 6.90.

For the L enantiomer of **9**, values of m.p. 174–176° and $[\alpha]_D$ −124.2° in chloroform have been reported[17].

*1-O-Acetyl-2,3,6-trideoxy-4-O-p-nitrobenzoyl-3-trifluoroacetamido-*D-ribo*-hexopyranose* (**10**). — To a cold (−25°) mixture of acetic anhydride (20 mL), acetic acid (15 mL), ethyl acetate (35 mL), and sulfuric acid (200 µl) was added the methyl glycoside **9** (1 g, 246 mmol). The reaction was complete (t.l.c., 4:1 ether–petroleum ether) after 4 h at −25° and 14 h at −13°. Aqueous sodium acetate was then added, and the mixture was evaporated. Toluene (three 10-mL portions) was added to and evaporated from the residue, which was then dissolved in dichloromethane. The solution was clarified by filtration, and then evaporated, to afford the amorphous title-compound **10** in theoretical yield (1.07 g) as a 2:1 mixture (as judged from ^{1}H-n.m.r. data) of the α and β anomers. This material proved pure enough for the subsequent elimination-reaction, and no attempts were undertaken to separate the two anomers; $\nu_{max}^{CHCl_3}$ 3435 (NH), 1738 (ester C=O, Amide I), 1533 (Amide II, NO_2), and 1350 cm^{-1} (NO_2); ^{1}H-n.m.r. (chloroform-*d*, partial data): δ 6.31 (1 H, br d, $J_{1,2}$ <1 Hz, $J_{1,2'}$ 4 Hz, H-1 α anomer), ~6.28 (1 H, dd, $J_{1,2}$ ~8 Hz, $J_{1,2'}$ ~3 Hz, H-1 β anomer), 2.15 (3 H, s, acetyl-H, α anomer), 2.08 (3 H, s, acetyl-H β anomer), 1.26 (3 H, d, $J_{5,6}$ 6 Hz, H-6 α anomer), 1.43 (3 H, d, $J_{5,6}$ 8 Hz, H-6 β anomer); *m/e* (rel. intensity): 434 (M^+, absent), 391 (2, M — Ac·), 390 (3, M — MeCHO), 375 (6, M — AcO·), 207 (58, M — HOAc — $O_2NC_6H_4CO_2H$), 192 (40, 207 — Me·), 150 (100, $O_2NC_6H_4CO^+$), 104 (30), 95 (23, methylpyrylium cation), 76 (18), and 43 (93, Ac^+).

Anal. Calc. for $C_{17}H_{17}F_3N_2O_8$ (434.33): C, 47.01; H, 3.95; N, 6.45. Found: C, 47.15; H, 4.03; N, 6.20.

*1,5-Anhydro-2,3,6-trideoxy-4-O-p-nitrobenzoyl-3-trifluoroacetamido-*D-ribo*-hex-1-enitol* (**11**). — A mixture of the 1-acetate **10** (1 g, 2.30 mmol) and silica gel (10 g) in toluene (100 mL) was boiled under reflux in a flask equipped with a Dean–Stark water separator. T.l.c. (4:1 ether–petroleum ether) indicated reaction to be complete after 1 h. The silica gel was filtered off and washed thoroughly with ethyl acetate (five 20-mL portions), and the filtrates were combined, washed successively with saturated aqueous sodium hydrogencarbonate (two 50-mL portions) and water, dried, and evaporated. Kugelrohr distillation at 180° (bath temp.)/30 mtorr of the residue furnished **11** as a colorless glass; yield 560 mg (65%), $[\alpha]_D^{28}$ +87° (*c* 0.8, chloroform); $\nu_{max}^{CHCl_3}$ 3438 (NH), 1736 (ester C=O, Amide I), 1650 (C=C), 1532 (Amide II, NO_2), and 1353 cm^{-1} (NO_2); ^{1}H-n.m.r. (chloroform-*d*): δ 8.40–8.05

(4 H, m, aryl-H), 6.57 (1 H, d, $J_{1,2}$ 5.5 Hz, H-1), 6.43 (1 H, br d, $J_{3,NH}$ 8.0 Hz, NH-3), 5.25 (1 H, dd, $J_{3,4}$ 4.5 Hz, $J_{4,5}$ 9 Hz, H-4), 5.10–4.70 (2 H, m, H-2,3), 4.20 (1 H, dq, $J_{5,6}$ 6.5 Hz, H-5), and 1.39 (3 H, d, H-6); *m/e* rel. intensity: 374 ($M^{+\cdot}$, absent), 207 (40, M — $O_2NC_6H_4CO_2H$), 192 (96, 207 — Me·), 150 (100, $O_2NC_6H_4CO^+$), 120 (17), 105 (28), 95 (16, methylpyrylium cation), 76 (19), and 69 (12, CF_3^+).

Anal. Calc. for $C_{15}H_{13}F_3N_2O_6$ (374.28): C, 48.14; H, 3.50. Found: C, 47.86; H, 3.79.

7-O-(2,3,6-Trideoxy-4-O-p-nitrobenzoyl-3-trifluoroacetamido-α-D-ribo-hexopyranosyl)daunomycinone (**3**). — A mixture of daunomycinone (115 mg, 0.29 mmol), the glycal **11** (310 mg, 0.83 mmol), and *p*-toluenesulfonic acid monohydrate (10 mg) in benzene (15 mL) was stirred for 48 h at 25°, after which time, t.l.c. (1 : 1 benzene–ethyl acetate) revealed the presence of a new, major product (R_F 0.5) migrating as a red zone. The solvent was evaporated off, and the remaining syrup chromatographed on silica gel with the t.l.c. solvent system as eluant, to afford, after recrystallization from chloroform–hexane, the title compound **3**, whose analysis indicated it to be a monohydrate; yield 195 mg (85%, based on daunomycinone), m.p. 148–150°, $[\alpha]_D^{28}$ +250° (*c* 0.03, chloroform); $\lambda_{max}^{CHCl_3}$ 234 (ε_{mM} 28.4), 254 (27.8), 286 (17.0), 478 (15.9), 498 (15.3), and 533 nm (9.9); $\nu_{max}^{CHCl_3}$ 3375 (OH, NH), 1728 (*C*- and *O*-acyl C=O, Amide I), 1621 and 1580 (chelated quinone), 1541 (Amide II, NO_2), and 1350 cm^{-1} (NO_2); ^{1}H-n.m.r. (chloroform-*d*, partial data): δ 5.62 (1 H, dd, $J_{1,2}$ 3, $J_{1,2'}$ ~1 Hz, H-1'), 5.51 (1 H, dd, H-4'), 4.10 (3 H, s, OMe), 2.44 (3 H, s, $COCH_3$), and 1.39 (3 H, d, $J_{5,6}$ 5 Hz, H-6'); X-ray powder diffraction data: 13.95 w, 11.59 s, 8.93 m, 8.23 vs (3), 7.41 vw, 6.83 w, 6.51 m, 5.70 vs (2), 5.47 w, 4.81 m, 4.31 m, 4.18 w, and 3.96 vs (1).

Anal. Calc. for $C_{36}H_{31}F_3N_2O_{14} \cdot H_2O$ (790.66): C, 54.69; H, 4.21; N, 3.54. Found: C, 54.36; H, 4.56; N, 3.27.

7-O-(3-Amino-2,3,6-trideoxy-α-D-ribo-hexopyranosyl)daunomycinone hydrochloride (**4**). — A solution of the protected glycoside **3** (100 mg, 0.13 mmol) in oxolane (12 mL) and 0.1M aqueous sodium hydroxide (12 mL) was kept for 2 h at 0°, after which time, t.l.c. (1 : 1 methanol–acetone) revealed the presence of a new product (R_F 0.6). The pH was then adjusted to 6 with 0.1M hydrochloric acid, and the oxolane was distilled off *in vacuo* (bath temp. 25°). The aqueous solution remaining was rendered alkaline (pH 9), and extracted with chloroform (five 20-mL portions). The combined extracts were dried (sodium sulfate), and evaporated, to afford a red solid that was dissolved in chloroform (30 mL), and the solution treated with a stoichiometric amount of hydrogen chloride in ethanol. Ether was added, and the resulting precipitate was collected, to give the title compound **4** as a red, amorphous (diffuse X-ray powder diffraction pattern) solid; yield 48 mg (63%), m.p. 161–163° (dec.), $[\alpha]_D^{22}$ +522° (*c* 0.03, methanol); λ_{max}^{MeOH} 236 (ε_{mM} 24.4), 254 (20.6), 289 (8.0), 484 (10.3), and 529 nm (5.7); ν_{max}^{KBr} 3420 (OH, NH), 1712 (*C*-acetyl), 1625 and 1580 (chelated quinone).

Anal. Calc. for $C_{27}H_{29}NO_{10} \cdot HCl \cdot 2\,H_2O$ (600.03): C, 54.05; H, 5.71; N, 2.33. Found: C, 54.47; H, 5.78; N, 2.13.

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REFERENCES

1 S. K. CARTER, *J. Natl. Cancer Inst.*, 55 (1975) 1265–1274; A. DI MARCO, F. ARCAMONE, AND F. ZUNINO, in J. W. CORCORAN AND F. E. HAHN (Eds.), *Antibiotics*, Vol. III, Springer Verlag, New York, 1975, pp. 101–128.
2 M. GHIONE, *Cancer Chemother. Pharmacol.*, 1 (1978) 25–34.
3 D. W. HENRY, *Am. Chem. Soc. Symp. Ser.*, 30 (1976) 15–57.
4 F. ARCAMONE, *Lloydia*, 40 (1977) 45–66.
5 H. S. EL KHADEM AND D. L. SWARTZ, *Carbohydr. Res.*, 65 (1978) c1–c2, and papers cited therein.
6 G. MATHÉ AND L. M. VAN PUTTEN, *Cancer Chemother. Pharmacol.*, 1 (1978) 5–13.
7 E.-F. FUCHS, D. HORTON, W. WECKERLE, AND B. WINTER, *J. Antibiot.*, 32 (1979) 229–238, and earlier papers, cited therein.
8 M. ISRAEL, E. J. MODEST, AND E. FREI, *Cancer Res.*, 35 (1975) 1365–1368.
9 F. ARCAMONE, L. BERNARDI, P. GIARDINO, B. PATELLI, A. DI MARCO, A. M. CASAZZA, G. PRATESI, AND P. REGGIANI, *Cancer Treat. Rep.*, 60 (1976) 829–834; A. DI MARCO, A. M. CASAZZA, F. GIULIANI, G. PRATESI, F. ARCAMONE, L. BERNARDI, G. FRANCHI, P. GIARDINO, B. PATELLI, AND S. PENCO, *ibid.*, 62 (1978) 375–380.
10 E.-F. FUCHS, D. HORTON, W. WECKERLE, AND E. WINTER-MIHALY, *J. Med. Chem.*, 22 (1979) 406–411; T.-M. CHEUNG, D. HORTON, AND W. R. TURNER, *Am. Chem. Soc., Chem. Soc. Jpn. Joint Conf.*, (1979) CARB-76.
11 D. HORTON AND W. WECKERLE, *Carbohydr. Res.*, 46 (1976) 227–235.
12 D. HORTON AND W. WECKERLE, *Carbohydr. Res.*, 44 (1975) 227–240.
13 H. H. BAER AND F. F. Z. GEORGES, *Carbohydr. Res.*, 55 (1977) 253–258.
14 I. PELYVÁS, F. SZTARICSKAI, L. SZILÁGYI, R. BOGNÁR, AND J. TAMÁS, *Carbohydr. Res.*, 68 (1979) 321–330.
15 W. W. LEE, H. Y. WU, J. J. MARSH, JR., C. W. MOSHER, E. M. ACTON, L. GOODMAN, AND D. W. HENRY, *J. Med. Chem.*, 18 (1975) 767–768.
16 F. SZTARICSKAI, I. PELYVÁS, L. SZILÁGYI, R. BOGNÁR, J. TAMÁS, AND A. NESZMÉLYI, *Carbohydr. Res.*, 65 (1978) 193–200.
17 F. ARCAMONE, A. BARGIOTTI, G. CASSINELLI, S. PENCO, AND S. HANESSIAN, *Carbohydr. Res.*, 46 (1976) c3–c5.
18 K. HEYNS, M.-J. LIM, AND J. I. PARK, *Tetrahedron Lett.*, (1976) 1477–1480; J. BOIVIN, C. MONNERET, AND M. PAÏS, *Abstr. VIIIe Journées des Glucides, Chamerolles, France*, December 17–20, 1978, abstr. 8.
19 J. BOIVIN, M. PAÏS, AND C. MONNERET, *Carbohydr. Res.*, 64 (1978) 271–278.
20 D. HORTON, R. J. SORENSON, AND W. WECKERLE, *Carbohydr. Res.*, 58 (1977) 125–138.