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OXYHALOGENATION OF GLYCALS FOR THE SYNTHESIS OF ANTI-TUMOR-ACTIVE 2′-HALO DAUNORUBICIN ANALOGS*

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ABSTRACT

Alkoxyhalogenation of L-rhamnal diacetate with daunomycinone and *N*-iodosuccinimide afforded 37% of 7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-2-iodo-α-L-mannopyranosyl)daunomycinone (**4**, NSC 331,962) and 7% of the β-L-*gluco* analog (NSC 353,457); a similar procedure with L-fucal diacetate gave 77% of 7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-2-iodo-α-L-talopyranosyl)daunomycinone (NSC 327,472). Compound **4** showed high activity (T/C 247) and low toxicity in the P-388 lymphocytic leukemia screen in mice.

INTRODUCTION

Doxorubicin (adriamycin, **2**) is used in the treatment of many forms of cancer, and is considered superior to daunorubicin (daunomycin, **1**), the initial anthracycline antibiotic to arouse significant interest for cancer chemotherapy[2]. Both of these fermentation products manifest the adverse side-effects characteristic of most antineoplastic drugs, associated with selective toxicity toward cells in rapid turnover, as well as a cumulative, dose-related cardiotoxicity; they are also readily hydrolyzed *in vivo* to afford the sugar component and the aglycon. These components display no antitumor activity, and the aglycon is very insoluble. For these reasons, there is much interest in the development of active analogs in which these drawbacks are overcome or minimized.

Our research program toward these ends has focused on the effect of selective structural and stereochemical modification of these anthracycline antibiotics, especially in the sugar component, through synthesis of a range of sugar analogs, their glycosidic coupling to both natural and synthetic aglycons, and biological evaluation of the resulting conjugates[3,4]. The results have demonstrated that the stereochemistry of the sugar is of profound importance, but that the 3′-amino group is not essential for activity—arguments for its putative key role in drug action not-

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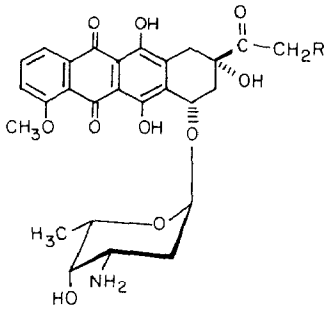


1 R = H, daunorubicin

2 R = OH, doxorubicin (adriamycin)

withstanding—as various 3′-hydroxy analogs have demonstrated good activity coupled with decreased toxicity[4,5].

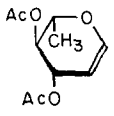
Early suggestions[2] that substituents at the 2′ position lead to inactivation have, perhaps, stifled thorough investigation of the role of groups at this position. Substitution at C-2′ could offer an effective means for controlling the susceptibility of the glycosidic linkage to hydrolysis as well as offering possibilities for further functional-group modification, and this rationale provided the basis for the current synthesis and evaluation of 2′-halo analogs. The inductive effect of the halogen should greatly stabilize these compounds toward glycoside hydrolysis *in vivo*, permit their longer persistence without degradation at the target site, and prevent liberation of the free aglycon in the tissues. It is shown here that introduction of an axial iodine atom at C-2′ is consistent with retention and indeed enhancement of antitumor activity for the 4′-epi analogs, as demonstrated in the murine P-388 lymphocytic leukemia, L-1210 lymphoid leukemia, B-16 melanocarcinoma, and Lewis lung carcinoma assays; appropriate design of suitable molecules offers promise for effective, new-generation anthracycline analogs obtained by semisynthetic or totally synthetic routes.

The chemical basis of the syntheses is modeled after the alkoxyhalogenation of glycal derivatives by use of *N*-iodosuccinimide and an alcohol, as demonstrated by Thiem *et al.*[6] with simple alcohols, and the results indicate that the benzylic alcohol group of an anthracyclinone may be coupled effectively by this approach.

RESULTS AND DISCUSSION

Chemical synthesis. — 2-Deoxy-2-halogenoglycosides are useful intermediates for the synthesis of 2-deoxyglycosides, and several methods for their synthesis have been developed[7]. The present synthesis is based on a method[6] employing an alcohol, a glycal, and *N*-iodosuccinimide, and it is shown that a complex alcohol, in this instance an anthracyclinone, may be effectively utilized in this reaction.

L-Rhamnal diacetate (**3**) reacted with daunomycinone in the presence of *N*-iodosuccinimide to afford mainly the product of diaxial opening of an intermediate iodonium ion, *i.e.*, the α-L-*manno* glycoside **4**. The ^{1}H-n.m.r. spectrum of **4** showed that the sugar ring exists in the $^1C_4$ conformation ($J_{3',4'}$ 9.5, $J_{4',5'}$ 9.9 Hz) and that H-1′ and H-2′ are equatorial ($J_{1',2'}$ 1.3, $J_{2',3'}$ 4.3 Hz). A second product appeared to be compound **5** with *trans*-diequatorial substituents at C-1′ and C-2′. The $J_{1',2'}$ (9.2 Hz) and $J_{2',3'}$ (11.0 Hz) values confirmed the assigned β-L-*gluco* configuration.



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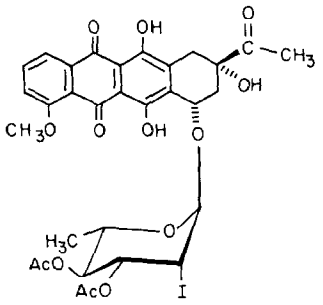
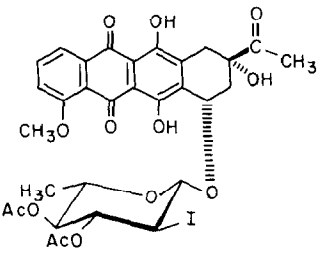
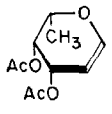


4, NSC 331,962



5, NSC 353,457



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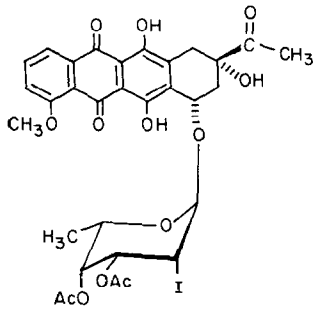

7, NSC 327,472

Only one product (**7**) was isolated from the reaction of 3,4-di-*O*-acetyl-L-fucal (**6**) with daunomycinone and *N*-iodosuccinimide. The α-L-*talo* configuration of **7** was confirmed by the $J_{1',2'}$ value of 1.5 Hz, indicating the equatorial orientation of both protons. Assignment of the equatorial orientation to H-2′ is also supported by the $J_{2',3'}$ value of 5.0 Hz.

The observed ^{13}C-n.m.r. spectra (Table I) for all compounds were in full agreement with proposed structures and with the ^{13}C-n.m.r. spectra of the respective methyl 2-deoxy-2-iodoglycosides.

Biological activity[10]. — Compound **4** (NSC 331,962) displayed high *in vivo*

TABLE I

^{13}C-N M R CHEMICAL SHIFT DATA (δ) FOR COMPOUNDS **4**, **5**, AND **7**[a]

C-Atom	**4**	**5**	**7**	*C-Atom*	**4**	**5**	**7**
1	119.9	119.7	119.9	9	76.5	76.3	76.4
2	135.7	135.5	135.7	10	33 3	34.0[c]	33.2
3	118.5	118.5	118 6	13	211.4	212.0	211.4
4	161.1	161.1	161.1	14	24 6	24 7	24.6
6	156.6	156.5	156.3	OMe	56 7	56.6	56 7
11	156.6	155 6	155.6	1′	104 8	103.2	106.2
5	186.9	186.8	186 9	2′	29.6	30.1	21.0
12	186 7	186.5	186.8	3′	69.1	75.5	65.8
4a	120.8	120 8	120.9	4′	72 5	74.1	68 1
5a	111.5	111.4	111.6	5′	68 3	70 3[b]	66.0
11a	111 5	111 0	111 5	Me-5′	17 5	17.1	16.1
6a	135.5	135.8	135.5	OAc	20.9	20.6	21.0
10a	134.4	135.5	134 3	OAc	20 8	20.5	20.8
12a	133 1	133.2	133.2	C=O	169 7	169 6	170.4
7	70.9	70.4[b]	70.9	C=O	169.6	169.5	169.4
8	35.3	34 2[c]	35.1				

[a]For solutions in chloroform-*d*. Chemical-shift assignments are based on off-resonance decoupling plus single-frequency, selective heteronuclear decoupling and comparison with literature values[8,9]. Assignments bracketed are not specifically differentiated. [b,c]Assignments may be interchanged.

activity in the murine P-388 lymphocytic leukemia test*, showing T/C 247 (50 mg/kg) in an initial test that was confirmed in a second assay that showed T/C 208 (25.00 mg/kg). The compound also displayed activity against L-1210 lymphoid leukemia (T/C 196 at 25 mg/kg, total 9 intraperitoneal injections, starting on the first day) and against Lewis lung carcinoma (T/C 187 at 25 mg/kg, total 9 intravenous injections, starting on the first day). Furthermore, the 2′-iodo-4′-epi analog **4** showed significant activity in the B-16 melanoma assay (T/C 218 at 25 mg/kg, total 9 intraperitoneal injections, starting on the first day).

Compound **7** (NSC 327,472) showed activity in the P-388 assay in one test (T/C 172 at a dose of 12.5 mg/kg), but this was not confirmed in two subsequent tests at does up to 25 mg/kg. To clarify these findings, the P-388 test was repeated by a different screener (Adria Laboratories, Inc., Dublin, Ohio. Test conditions: 1 intraperitoneal injection on day 1). At the highest dose tested (150 mg/kg), the recorded T/C value was 162. Compound **5** (NSC 353,457) did not display activity in the P-388 test* at doses up to 50 mg/kg.

EXPERIMENTAL

General methods. — T.l.c. was performed on Silica Gel 60 F_{254} (Merck), and column chromatography with Silica Gel 60 (230–400 mesh) (Merck). Melting points

*Data obtained under the auspices of the U S. National Cancer Institute, Division of Cancer Treatment, Drug Research and Development Branch. Test conditions: 3 injections (intraperitoneal) on days 5, 9, and 13.

were determined with a Thomas–Hoover apparatus and are uncorrected. I.r. spectra were recorded with a Perkin–Elmer 457 grating spectrophotometer. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. N.m.r. spectra were recorded for solutions in chloroform-*d* (internal Me_4Si) with a Bruker WP-200 (^1H, 200 MHz; ^{13}C, 50 MHz) or Bruker WP-80 (^{13}C, 20 MHz) instrument. N.m.r. spectra were recorded by Drs. C. Cottrell and O. Mols. Elemental analyses were performed by Dr. O. Mols and W. N. Rond.

7-O-(3,4-Di-O-acetyl-2,6-dideoxy-2-iodo-α-L-mannopyranosyl)daunomycinone (**4**, NSC 331,962) *and 7-O-(3,4-di-O-acetyl-2,6-dideoxy-2-iodo-β-L-glucopyranosyl)daunomycinone* (**5**, NSC 353,457). — Daunomycinone (604.4 mg, 1.52 mmol) and 3,4-di-*O*-acetyl-L-rhamnal (**3**; 355 mg, 1.57 mmol) were dissolved in a mixture of dry acetonitrile (12 mL) and oxolane (5 mL). The mixture was cooled to 0° and vigorously stirred while *N*-iodosuccinimide (512 mg, 2.27 mmol) was added. The resulting mixture was stirred for 15 min at 0° and then kept overnight at room temperature. T.l.c. (4:1 benzene–acetone) revealed one major and a minor, less-polar component. Dichloromethane (25 mL) was added and the resulting solution was washed twice with 10% aqueous sodium thiosulfate (20 mL) and then water (twice, 30 mL), dried ($MgSO_4$), filtered, and evaporated under diminished pressure. The product was immediately chromatographed on silica gel (8:1 benzene–acetone) to give **4** (412.3 mg, 37%), m.p. 142–144° (from acetone–hexane), $[\alpha]_D^{27}$ +69.9° (*c* 0.02, chloroform); ν_{max}^{KBr} 3490 (OH), 1747 (O-acetyl), 1714 (C-acetyl), 1618 and 1573 (chelated quinone), 1377, 1284, 1235, 1212, 1118, 1069, 1049, and 992 cm^{-1}; ^1H-n.m.r.: δ 13.99, 13.23 (2 s, each 1 H, HO-6,11), 8.03 (dd, 1 H, $J_{1,2}$ 7.7, $J_{1,3}$ 0.9 Hz, H-1), 7.78 (apparent t, 1 H, H-2), 7.39 (dd, 1 H, $J_{2,3}$ 8.6 Hz, H-3), 5.77 (bs, 1 H, H-1′), 5.25 (m, 1 H, H-7), 5.18 (t, 1 H, H-4′), 4.59 (dd, 1 H, $J_{1',2'}$ 1.3, $J_{2',3'}$ 4.3 Hz, H-2′), 4.36 (dd, 1 H, $J_{3',4'}$ 9.5 Hz, H-3′), 4.13 (dq, 1 H, $J_{4',5'}$ 9.9, $J_{5',6'}$ 6.5 Hz, H-5′), 4.09 (s, 3 H, OMe), 4.03 (s, 1 H, HO-9), 3.24 (dd, 1 H, $J_{8e,10e}$ 1.5 Hz, H-10*e*), 2.92 (d, 1 H, $J_{10e,10ax}$ *18.9 Hz, H-10ax*), 2.42 (s, 3 H, H-14), 2.33 (apparent dt, 1 H, $J_{8e,8ax}$ 15.7 Hz, H-8*e*), 2.17 (m, 1 H, H-8*ax*), 2.06, 2.03 (s, 3 H, OAc), 1.29 (d, 3 H, H-6′).

Anal. Calc. for $C_{31}H_{31}IO_{13}$ (738.489): C, 50.42; H, 4.23; I, 17.18. Found: C, 50.20; H, 4.37; I, 17.39.

The minor, less-polar component (**5**, NSC 353,457) was rechromatographed (10:1 toluene–acetone) to afford 80 mg (7%) of a red solid that was pure by t.l.c. Crystallization from chloroform–acetone–hexane gave **5** (45 mg), m.p. 184–188°, $[\alpha]_D^{22}$ +308° (*c* 0.02, chloroform); ν_{max}^{KBr} 3540 (OH), 1752 (O-acetyl), 1712 (C-acetyl), 1620 and 1580 cm^{-1} (H-bonded quinone); ^1H-n.m.r.: δ 14.14, 13.23 (2 s, each 1 H, HO-6,11), 8.00 (dd, 1 H, $J_{1,2}$ 7.7, $J_{1,3}$ 1.1 Hz, H-1), 7.76 (apparent t, 1 H, H-2), 7.39 (dd, 1 H, $J_{2,3}$ 8.6 Hz, H-3), 5.56 (apparent t, 1 H, $J_{7,8ax}$ + $J_{7,8e}$ 5.8 Hz, H-7), 5.28 (dd, 1 H, $J_{2',3'}$ 11.0, $J_{3',4'}$ 9.0 Hz, H-3′), 5.16 (d, 1 H, $J_{1',2'}$ 9.2 Hz, H-1′), 4.61 (t, 1 H, H-4′), 4.43 (s, 1 H, HO-9), 4.09 (s, 3 H, OMe), 3.84 (dd, 1 H, H-2′), 3.57 (dq, 1 H, $J_{4',5'}$ 9.6, $J_{5',6'}$ 6.2 Hz, H-5′), 3.24 (dd, 1 H, $J_{8e,10e}$ <1.5 Hz, H-10*e*), 3.02 (d, 1 H, $J_{10ax,10e}$ 19.1 Hz, H-10*ax*), 2.62 (m, 1 H, H-8*e*), 2.43 (s, 3 H, H-14), 2.13–2.02 (m, 1 H, H-8*ax*), 2.07, 2.00 (s, 3 H, OAc), 0.97 (d, 3 H, H-6′).

Anal. Calc. for $C_{31}H_{31}IO_{13}$ (738.489): C, 50.42; H, 4.23. Found: C, 50.58; H, 4.42.

7-O-(3,4-Di-O-acetyl-2,6-dideoxy-2-iodo-α-L-talopyranosyl)daunomycinone (**7**, NSC 327,472). — To a vigorously stirred solution of daunomycinone (516 mg, 1.3 mmol) and 3,4-di-*O*-acetyl-L-fucal (**6**; 352.4 mg, 1.65 mmol) in dry acetonitrile (~10 mL) and oxolane (5 mL) at 0° (ice bath) was added *N*-iodosuccinimide (462.9 mg, 2.06 mmol). After 15 min, the ice bath was removed and the mixture was stirred overnight at room temperature and then processed as in the previous reaction. The products were combined with those of an exploratory reaction (from 64 mg of daunomycinone) and chromatographed on a column of silica gel (25 g) with 4:1 benzene–acetone; yield, 737 mg (77%); m.p. 131°, $[\alpha]_D^{25}$ +64.9° (*c* 0.02, chloroform); ν_{max}^{KBr} 3510 (OH), 1748 (O-acetyl), 1720 (C-acetyl), 1621 and 1583 (chelated quinone), 1450, 1420, 1380, 1289, 1240, 1120, 1092, 1040, 995, 825, 798, and 771 cm^{-1}; ^1H-n.m.r.: δ 14.00, 13.25 (2 s, each 1 H, HO-6,11), 8.04 (dd, 1 H, $J_{1,2}$ 7.7, $J_{1,3}$ 1.3 Hz, H-1), 7.79 (apparent t, 1 H, H-2), 7.40 (dd, 1 H, $J_{2,3}$ 7.3 Hz, H-3), 5.92 (bs, 1 H, H-1'), 5.24 (m, 2 H, H-4',7), 4.71 (dd, 1 H, $J_{2',3'}$ 5.0, $J_{3',4'}$ 3.5 Hz, H-3'), 4.41 (qd, 1 H, H-5'), 4.34 (dd, 1 H, $J_{1',2'}$ 1.5 Hz, H-2'), 4.09 (s, 3 H, OCH_3), 3.97 (s, 1 H, HO-9), 3.23 (dd, 1 H, $J_{8e,10e}$ 1.7 Hz, H-10*e*), 2.94 (d, 1 H, $J_{10e,10ax}$ 18.9 Hz, H-10*ax*), 2.41 (s, 3 H, H-14), 2.33 (m, 1 H, H-8*e*), 2.14 (m, 1 H, H-8*ax*), 2.21, 2.03 (s, 3 H, OAc), 1.27 (d, 3 H, $J_{5',6'}$ 6.45 Hz, H-6').

Anal. Calc. for $C_{31}H_{31}IO_{13} \cdot H_2O$ (756.593): C, 49.21; H, 4.40; I, 16.77. Found: C, 49.27; H, 4.83; I, 17.37.

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