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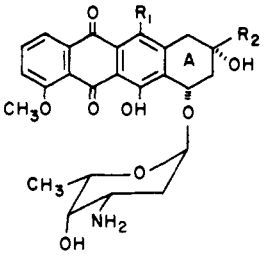
## Structures of Novel Anthracycline Antitumor Antibiotics from *Micromonospora peucetica*

*Sir:*

In our continuing search for new natural[1,2] and semisynthetic[3] analogues of the useful anticancer drugs daunorubicin (**1a**)[4] and doxorubicin (**1b**),[5] we have examined the fermentation broths of *Micromonospora peucetica* n. sp. This has given an anthracycline complex whose glycosidic constituents represent a novel structural class within the family of doxorubicin related anthracyclines. In this communication we report the isolation and structure determination of the new, biologically active anthracyclines 11-deoxydaunorubicin (**2**), 11-deoxydoxorubicin (**3**), 11-deoxy-13-dihydrodaunorubicin (**4**), and 11-deoxy-13-deoxodaunorubicin (**5**).

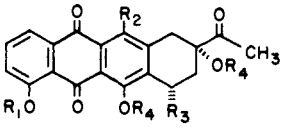


**1a**: $R_1$ = OH; $R_2$ = $COCH_3$
**1b**: $R_1$ = OH; $R_2$ = $COCH_2OH$
**2**: $R_1$ = H; $R_2$ = $COCH_3$
**3**: $R_1$ = H; $R_2$ = $COCH_2OH$
**4**: $R_1$ = H; $R_2$ = $CHOHCH_3$
**5**: $R_1$ = H; $R_2$ = $CH_2CH_3$

Purification of the anthracycline complex (6 g), isolated in the usual way,[2] on a silica gel column[6] gave **2** (0.4 g) ($C_{27}H_{29}NO_9 \cdot HCl$[7], mp 175–176 °C dec, $[\alpha]^{23}_D$ +139°), **3** (0.6 g) ($C_{27}H_{29}NO_{10} \cdot HCl$, mp 171–173 °C dec, $[\alpha]^{23}_D$ +111°), **4** (0.2 g) ($C_{27}H_{31}NO_9 \cdot HCl$, mp 163–164 °C dec, $[\alpha]^{23}_D$ +107°), and **5** (0.2 g) ($C_{27}H_{31}NO_8 \cdot HCl$, mp 142–146 °C dec).

The UV and visible spectra [$\lambda_{max}$ (MeOH) 228, 260, 418 nm] suggested the presence of the same hydroxyanthraquinone chromophore in all four compounds,[8] while the IR (KBr) indicated the presence[9] of both nonhydrogen bonded (1670 $cm^{-1}$) and hydrogen bonded (1625 $cm^{-1}$) quinone carbonyl groups and an additional carbonyl function in **2** (1710 $cm^{-1}$) and **3** (1725 $cm^{-1}$).

Mild acid hydrolysis (0.2 N HCl, 100 °C, 1 h) of the four glycosides afforded the same amino sugar, identified as daunosamine[10] by direct comparison with an authentic sample, and four aglycones differing only in the side chain. Acid hydrolysis of **2** yielded the aglycone **6**: $C_{21}H_{18}O_7$; mp 213–215 °C; IR (KBr) 1710 (CO), 1670 and 1620 $cm^{-1}$ (quinone bands); $^1H$ NMR ($CDCl_3$) $\delta$ 2.42 (s, 3 H, $COCH_3$), 4.02 (s, 3 H, $ArOCH_3$), 5.33 (br, 1 H, C-7), 7.25–7.73 (m, 4 H, ArH), 13.60 (s, 1 H, ArOH). Upon acetylation ($Ac_2O$, pyr), **6** gave the corresponding tri-*O*-acetyl derivative **7**: $C_{27}H_{24}O_{10}$; mp 120–124 °C dec; IR (KBr) 1775 (phenolic Ac), 1735–1725 $cm^{-1}$ (aliphatic Ac and CO); $^1H$ NMR ($CDCl_3$) $\delta$ 2.05 and 2.25 (two s, 9 H, C-7 OAc, C-9 OAc, and $COCH_3$), 2.48 (s, 3 H, ArOAc), 6.45 (dd, 1 H, C-7), 7.30–8.00 (m, 4 H, ArH). This confirmed the presence of one phenolic OH and two OH's on the alicyclic ring.



**6**: $R_1$ = $CH_3$, $R_2$ = $R_4$ = H, $R_3$ = OH
**7**: $R_1$ = $CH_3$, $R_2$ = H, $R_3$ = OAc, $R_4$ = Ac
**8**: $R_1$ = $CH_3$, $R_2$ = $R_3$ = OH, $R_4$ = H
**9**: $R_1$ = $CH_3$, $R_2$ = $R_3$ = $R_4$ = H
**10**: $R_1$ = $R_2$ = $R_4$ = H, $R_3$ = OH

Thus the chemical and spectral properties of **6**, which indicated the presence of an anthraquinone chromophore bearing both an OH and an $OCH_3$, and an alicyclic ring with one acetyl group and two OH's, showed a close relationship to daunomycinone (**8**).[4] Furthermore Zn dust distillation of **6** and **8** gave the same benz[*a*]anthracene, establishing a linear tetracyclic system in **6**.

Catalytic hydrogenolysis (5% $Pd/BaSO_4$, $H_2O$, 1 h) of **2** afforded daunosamine and a new aglycone **9**: $C_{21}H_{18}O_6$; mp 186–189 °C; IR (KBr) 1710 (CO), 1670 and 1625 $cm^{-1}$ (quinone bands); $^1H$ NMR ($CDCl_3$) $\delta$ 2.25 (s, 3 H, $COCH_3$), 4.02 (s, 3 H, $ArOCH_3$), 7.10–7.90 (m, 4 H, ArH), 13.60 (s, 1 H, ArOH). This showed that the sugar moiety was attached to a benzylic position. Compound **9** can also be obtained by catalytic hydrogenolysis of **6** (5% $Pd/BaSO_4$, dioxane, 1 h).

Demethylation ($AlBr_3$, $CH_2Cl_2$, 40 °C, 1 h) of **6** yielded **10**: $C_{20}H_{16}O_7$; mp 140–142 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 2.39 (s, 3 H, $COCH_3$), 5.30 (br, 1 H, C-7), 7.25–7.80 (m, 4 H, ArH), 11.87 and 12.57 (two s, 2 H, ArOH). The presence in the IR (KBr) of **10** of nonhydrogen-bonded (1670 $cm^{-1}$) and hydrogen-bonded (1620 $cm^{-1}$) quinone carbonyl groups confirmed[10] that the methoxy and hydroxy substituents had to both be peri to the same quinone carbonyl group.

We next addressed the substitution pattern and the stereochemistry of ring A. The $^1H$ NMR and $^{13}C$ NMR spectra of **6**, when compared with those of daunomycinone (**8**),[4,11] indicated the presence of a quaternary carbon bearing hydroxyl and acetyl groups. The cis configuration of the two aliphatic OH's was shown by the preparation from **6** [$CH_3C(OCH_3)_2CH_3$, *p*-TsOH, dioxane, 72 h] of the corresponding *O*-isopropylidene derivative **11**: $C_{24}H_{22}O_7$; mp 84–88 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 1.18 and 1.55 (two s, 6 H, $>C(CH_3)_2$), 2.42 (s, 3 H, $COCH_3$), 4.01 (s, 3 H, $ArOCH_3$), 7.25–7.78 (m, 4 H, ArH), 13.10 (s, 1 H, ArOH). Acetylation of **11** yielded a mono-*O*-acetyl derivative (**12**): $C_{26}H_{24}O_8$; mp 157–160 °C; IR (KBr) 1770 (ArOAc), 1710 (CO), 1670 $cm^{-1}$ (quinone band); $^1H$ NMR ($CDCl_3$) $\delta$ 1.09 and 1.42 (two s, 6 H, $>C(CH_3)_2$), 2.38 and 2.50 (two s, 6 H, ArOAc, $COCH_3$), 4.00 (s, 3 H, $OCH_3$), 5.25 (br, 1 H, C-7), 7.25–7.90 (m, 4 H, ArH). When **12** was subjected to mild acid hydrolysis [$(CH_3)_2CO$, $H_2O$, $H_2SO_4$, 0.5 h] it gave **13**: $C_{23}H_{20}O_8$; mp

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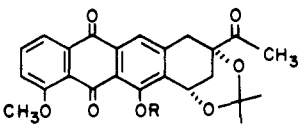
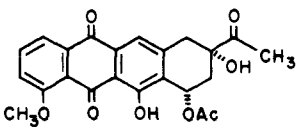


11 : R = H
12 : R = Ac



13

108–110 °C; IR (KBr) 1730 (C-7 OAc), 1710 (CO), 1670 and 1625 $cm^{-1}$ (quinone bands); $^1H$ NMR ($CDCl_3$) $\delta$ 2.08 (s, 3 H, C-7 OAc), 2.35 (s, 3 H, $COCH_3$), 4.04 (s, 3 H, $ArOCH_3$), 6.48 (br, 1 H, C-7), 7.21–7.85 (m, 4 H, ArH).

The formation of **13**, involving the acetate shift from the O at C-6 to the O at C-7,[18] established the relative position of the phenolic and benzylic hydroxyl groups and strongly suggested that **6** was 11-deoxydaunomycinone. Since the $\alpha$ configuration of its glycoside linkage was assigned on the basis of the C-1′ $^1H$ NMR (br s, $W_H$ = 7 Hz) at $\delta$ 5.26)[12] and $^{13}C$ NMR ($\delta$ 98.9)[11] signals in $Me_2SO$-$d_6$, **2** was most plausibly identified as 11-deoxydaunorubicin.

Direct chemical transformation of **2** into **3, 4,** and **5** completed the chemical structure work. Compound **3** was obtained by the known procedure[13] used to prepare doxorubicin (**1b**) from daunorubicin (**1a**), compound **4** by the side-chain carbonyl reduction ($NaBH_4$, $H_2O$) and **5** by reduction ($NaBH_4$, AcOH)[14] of the 13-tosylhydrazone of **2.**

A single-crystal X-ray anslysis of compound **7** was carried out to unequivocally confirm these assignments.

Crystals of aglycone **7,** which were suitable for single-crystal X-ray diffraction work, were grown from $CH_2Cl_2$–isooctane–MeOH. Preliminary X-ray photographs showed orthorhombic symmetry and accurate lattice constants, determined by a least-squares fit of 15 moderate $2\theta$ values, were $a$ = 5.438 (6), $b$ = 23.881 (42), and $c$ = 18.548 (42) Å. The systematic extinctions, presence of chirality, and an observed and calculated ($Z$ = 4) density of 1.40 g/$cm^3$ were uniquely accommodated by space group $P22_12_1$ (alternate setting) with one molecule of composition $C_{27}H_{24}O_{10}$ in the asymmetric unit. Intensity data were collected on a fully automatic four-circle diffractometer using graphite monochromated Mo K$\alpha$ radiation (0.71069 Å) and a variable speed, 1° $\omega$ scan. A total of 2511 unique diffraction maxima with $2\theta \leq 50°$ were measured and, after correction for Lorentz, polarization, and background effects, 2301 (92%) were judged observed [$|F_o| \geq 3\sigma(F_o)$]. A preliminary phasing model was arrived at using a multisolution weighted tangent formula approach.[15] Nonhydrogen atoms were refined anisotropically and hydrogen atoms, which were located on a difference electron density synthesis, were refined isotropically in a full-matrix least-squares treatment. The conventional crystallographic discrepancy index is 0.066 for the observed reflections.[15]

Figure 1 is a computer-generated perspective drawing of the final X-ray model less hydrogens. Only the relative configuration was determined in this experiment and the absolute configuration shown was chosen to agree with that of daunorubicin. The molecular geometry is very similar to that described previously.[16] The B, C, and D rings are planar and the A ring is in the half-chair conformation. The acetoxy substituent at C-9 is in a pseudoaxial position and the acetoxy substituent at C-7 has a cis relationship to it. In general bond distances and angles agree well with accepted values. See the paragraph at the end of this paper "Supplementary Material Available" for additional crystallographic details.

The four new glycosides were tested on an in vitro HeLa cell culture and showed $ID_{50}$'s ranging from 0.05 $\mu$g/mL for **2** to 0.44 $\mu$g/mL for **5.** When tested in vivo on P388 leukemia, **2** showed a T/C of 181 (100 mg/kg) and **3** a T/C of 245 (66 mg/kg). As a comparison doxorubicin has a T/C of 213 (6.6 mg/kg) in the same test.[17]

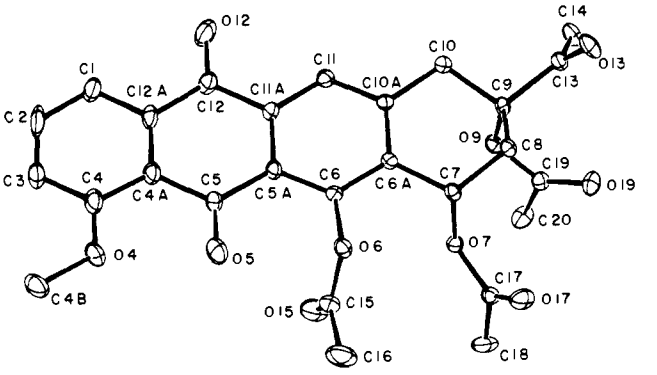



**Figure 1.** A computer-generated perspective drawing of the X-ray model of 11-deoxydaunorubicin aglycone triacetate (**7**). Hydrogens are omitted for clarity.

**Acknowledgment.** Part of the work at Cornell was supported by NIH grant NCI-CA-24487.

**Supplementary Material Available:** Final atomic positional and thermal parameters (Table I), bond distances (Table II), and bond angles (Table III) for the triacetate of 11-deoxydaunorubicin aglycone (4 pages). Ordering information is given on any current masthead page.

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(6) The crude complex was chromatographed on silica gel using a chloroform–methanol–water gradient. After elution of the aglycones, **5** and **2** were eluted with 89.5:10:0.5 chloroform–methanol–water.
(7) Satisfactory elemental analyses and/or field desorption mass spectra were obtained for all new compounds. Melting points are not corrected and $[\alpha]_D$'s were determined in 0.2% methanol solutions.
(8) $E^{1\%}_{1\,cm}$ of the new glycosides at $\lambda_{max}$ (MeOH) 228, 260, and 418 nm are, respectively, for **2,** 713, 415, 199; **3,** 645, 420, 193; **4,** 640, 410, 179; and **5,** 610, 395, 171.
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(17) Biological data were obtained at Instituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy, and are reported here by the courtesy of A. Di Marco and A. M. Casazza.
(18) The acetate shift from the O at C-6 to the O at C-7 was originally recorded on the 11-acetoxy analogue of **12** (G. Franceschi, unpublished data).

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