

SYNTHESIS AND ANTITUMOR ACTIVITY OF
3'-DEAMINO-3'-HYDROXYDOXORUBICIN
A FACILE PROCEDURE FOR THE PREPARATION OF
DOXORUBICIN ANALOGS

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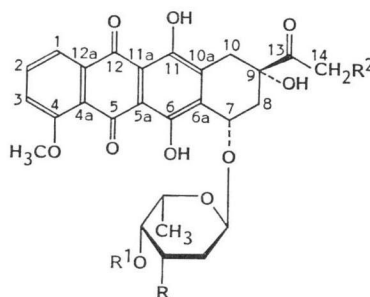
Two successful routes have been developed for preparation of 3'-deamino-3'-hydroxydoxorubicin (**11**), based on protection of the 14-hydroxyl group of the glycon by using *tert*-butylchlorodimethylsilane. The key intermediate, 14-*O-tert*-butyldimethylsilyl-7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy- α -*L*-lyxo-hexopyranosyl)adriamycinone (**9**), was successively deacetylated and desilylated in high yield to give the desired product **11**. This route constitutes a general method of access to glycon-modified doxorubicin analogs. Compound **11** showed high anti-tumor activity *in vivo* in the murine P388 lymphocytic leukemia assay.

Reports from this laboratory have shown that an amino group in the 3'-position is not essential for analogs of anthracycline antibiotics to exhibit significant antitumor activity¹⁻³. Results for the 3'-deamino analogs of doxorubicin reported here further reinforce this fact; comparative evaluations of these compounds could help in clarifying the biological role and effects of the 3'-amino group in the parent antibiotics daunorubicin (**2**) and doxorubicin (**1**).

Replacement of the amino function in daunorubicin (**2**) by hydroxyl¹ and in doxorubicin (**1**) by acetoxy³ generated analogs **3** (NSC 284,682) and **4** (NSC 307,990) that display high *in vivo* antitumor activity in a wide range of murine tests¹⁻³. The fully deprotected analog, 3'-deamino-3'-hydroxydoxorubicin³ (**11**) proved very difficult to prepare from its 3',4'-diacetate (**4**) in yields satisfactory for full characterization and biological evaluation.

The route that proceeds satisfactorily for transformation of daunorubicin into doxorubicin, namely bromination at C-14 followed by hydrolysis of the 14-bromide, led in the case of 3'-hydroxydaunorubicin (**3**) and its 3',4'-diacetate **5** to complex mixtures³. In another approach³, daunomycinone was first brominated at C-14, and then glycosylated at O-7. Hydrolysis gave the 3',4'-diacetylated doxorubicin analog **4**. However, procedures for *O*-deacetylation led to extensive glycosidic cleavage³. Similarly unsuccessful was a route employing 14-*O*-(*p*-anisylidiphenylmethyl)adriamycinone³.

The need to compare the fully deprotected target, 3'-deamino-3'-hydroxydoxorubicin (**11**), with doxorubicin (**1**) itself, together with the possibility of obtaining an analog having higher potency and activity than that of the diacetate^{2,3} **4** (T/C 269), prompted the present development of a new and



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|----------|-----------------------|----------------------|---------------------|
| 1 | R = NH ₂ , | R ¹ = H, | R ² = OH |
| 2 | R = NH ₂ , | R ¹ = H, | R ² = H |
| 3 | R = OH, | R ¹ = H, | R ² = H |
| 4 | R = OAc, | R ¹ = Ac, | R ² = OH |
| 5 | R = OAc, | R ¹ = Ac, | R ² = H |

efficient method for synthesis of unprotected, glycon-modified, doxorubicin analogs.

Chemical Synthesis

The key intermediate **9** was prepared by two different routes, from the precursors **4** or **7**. Silylation of adriamycinone (**6**) with *tert*-butylchlorodimethylsilane in *N,N*-dimethylformamide (DMF) in the presence of imidazole gave the 14-ether **7** as the principal product (82%). The ^{13}C NMR spectrum of **7** showed signals at 25.9, 18.5 and -5.4 ppm, confirming the presence of the *tert*-butyldimethylsilyl group. The aglycon **7** thus protected at 14-HO was then glycosylated with two molar equivalents of 3,4-di-*O*-acetyl-2,6-dideoxy- α -*L*-lyxo-hexopyranosyl chloride⁴⁾ (**8**) in the presence of yellow mercuric oxide, mercuric bromide and molecular sieves (4 Å) in dichloromethane for 24 hours at 25°C. One main product was formed which, after purification by crystallization, afforded the fully protected

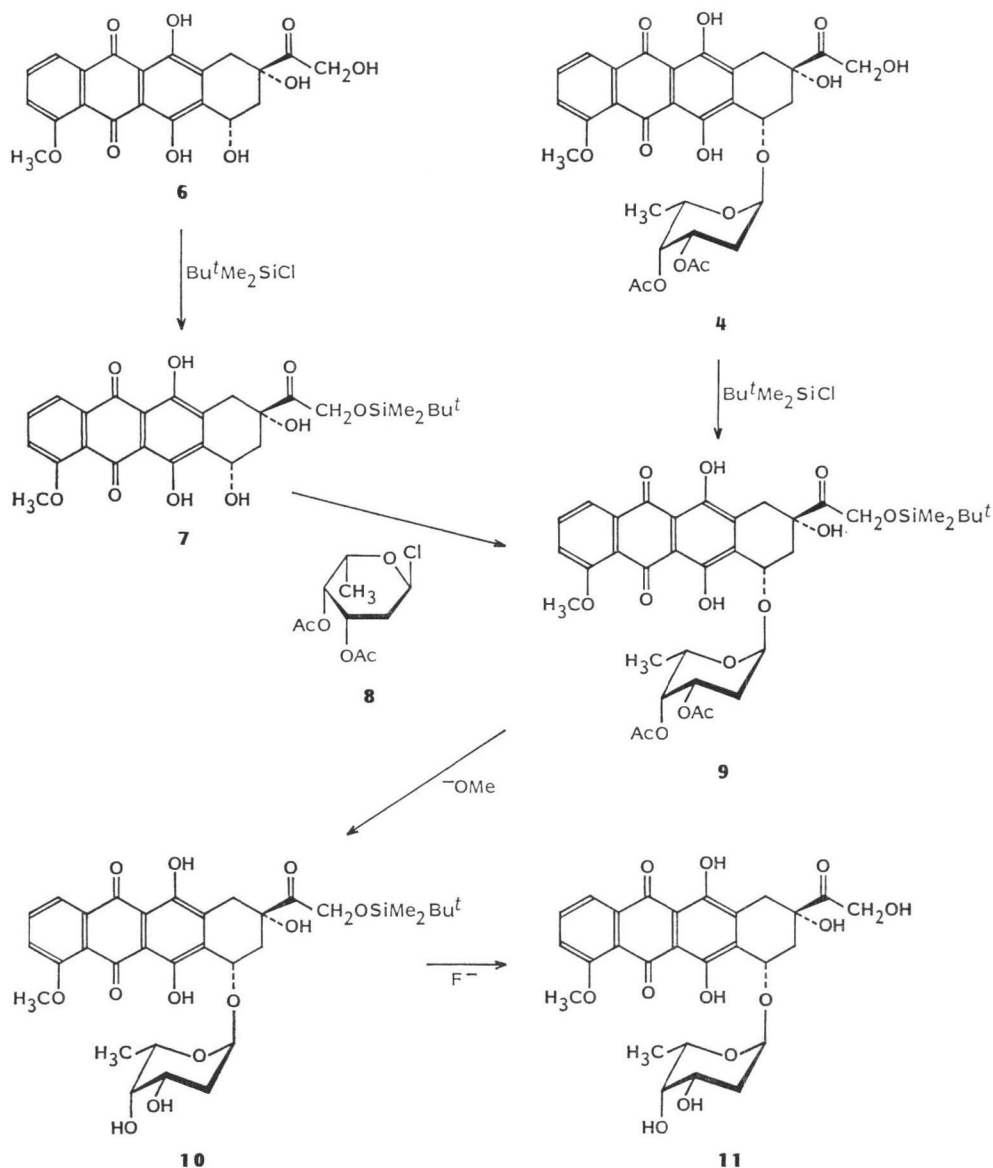


Table 1. ¹H NMR data for compounds 7, 9, 10 and 11.

Compound (solvent)	H-1 ($J_{1,2}$) ($J_{1,3}$)	H-2	H-3 ($J_{2,3}$)	H-1' ($J_{1',2'ax}$)	H-7	H-4' ($J_{3',4'}$)	H-3' ($J_{2'ax,3'}$) ($J_{2'eq,3'}$)	9-OH	H-14A ($J_{14A,14B}$)	H-14B	H-5' ($J_{4',5'}$)
7 (CDCl ₃)	7.97 (7.7) (0.8)	7.76	7.37 (8.4)	—	5.28	—	—	4.60	4.94 (19.5)	4.82	—
9 (CDCl ₃)	8.05 (7.8) (1.0)	7.79	7.40 (8.6)	5.60 (3.6)	←5.25→	(3.0)	5.05 (12.5) (5.2)	4.31	←4.90→	—	4.22 (~1.0)
10 (CDCl ₃)	8.01 (7.7) (1.0)	7.76	7.39 (8.3)	5.50	5.24	3.67 (2.8)	3.82 (9.3) (6.3)	4.49	←4.86→	—	4.04 (~1.0)
11 (C ₆ D ₆ N+D ₂ O)	7.98 (7.6)	7.71	7.40 (8.3)	5.73 (3.8)	5.35	3.38 (3.0)	4.39 (11.8) (5.0)	—	←5.29→	—	4.55 (~1.0)

	H-10eq ($J_{8eq,10eq}$)	H-10ax ($J_{10ax,10eq}$)	H-8eq ($J_{8eq,8ax}$)	H-8ax ($J_{7,8ax}$)	H-2'ax	H-2'eq ($J_{2'ax,2'eq}$)	H-6' ($J_{5',6'}$)	6-OH, 11-OH	OMe	SiCMe ₃	SiMe ₂
7	3.17 (1.7)	2.90 (18.8)	2.37 (14.8)	2.15 (4.8)	—	—	—	13.84, 13.13	4.07	0.95	0.14
9	3.25 (1.4)	3.00 (18.9)	←2.35~1.82→			—	1.20 (6.5)	14.0, 13.25	4.09	0.97	0.15
10	3.23 (1.5)	3.00 (19.0)	2.31 (14.9)	2.14 (4.0)	←2.00~1.74→		1.30 (6.3)	13.96, 13.24	4.06	0.94	0.12
11	3.48 (~1.7)	3.37 (18.9)	2.76 (14.8)	2.42 (5.0)	2.52	2.25 (12.5)	1.47 (6.5)	—	3.95	—	—

derivative **9** in 82% yield. In an alternative route, compound **9** was prepared in 85% yield by silylation of compound **4**. The α -L-configuration was readily assigned from the H-1' signal (δ 5.60, bd), which showed a $J_{1',2'}$ value of 3.6 Hz as the larger coupling constant. The chemical shift of C-1' (101.2 ppm) coincides with the C-1' shifts observed for the α -anomers of 14-esters of 3',4'-di-*O*-acetyl-3'-deaminodoxorubicin³¹. *O*-Deacetylation of compound **9** under standard conditions with sodium methoxide in methanol afforded in 88% yield the 3',4'-hydroxyl analog **10**, whose ¹H and ¹³C NMR spectra showed acetyl group signals to be absent. The deacetylation caused an upfield shift of the H-3' and H-4' signals from 5.05 and 5.25 ppm to 3.82 and 3.67 ppm, respectively. The product **10**, purified by simple crystallization, was desilylated in the final step. Tetrabutylammonium fluoride removed the *tert*-butyldimethylsilyl group completely in 1 hour at 25°C. Extraction and crystallization gave fully deprotected product **11** in 83% yield. Its ¹H NMR spectrum recorded for a solution in pyridine-*d*₅ was in full accord with the proposed structure. Characteristic values of the coupling constants $J_{1',2'_{ax}}$, $J_{2'_{ax},3'}$ and $J_{2'_{eq},3'}$ of 3.8, 11.8 and 5.0 Hz, respectively, clearly confirmed the configuration of the carbohydrate portion.

Biological Activity

Assay of 3'-deamino-3'-hydroxydoxorubicin (**11**) *in vivo* in the P388 lymphocytic leukemia system, with a single ip injection on the first day, showed the highest T/C value (462) at 25 mg/kg. The compound also remained active (T/C 142) at the lowest dose tested (3.12 mg/kg). From these results it is thus evident that replacement of the amino group in doxorubicin by hydroxyl leads to a decrease in toxicity accompanied by an increase in activity, at least with respect to the P388 assay.*

Comparison of the P388 activities of 7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy- α -L-*lyxo*-hexopyranosyl)-adriamycinone³¹ (**4**, NSC 307,990, T/C 269 at 50 mg/kg) and of 7-*O*-(2,6-dideoxy- α -L-*lyxo*-hexopyranosyl)daunomycinone¹³ (**3**, NSC 284,682, T/C 192 at 200 mg/kg) with the present results for 7-*O*-(2,6-dideoxy- α -L-*lyxo*-hexopyranosyl)adriamycinone (**11**) indicate that introduction of a hydroxyl group at C-14 and deprotection of the hydroxyl groups at C-3' and C-4' leads successively to substantial increases in activity and potency.

Experimental

TLC was performed on precoated plastic sheets (0.22 mm) and glass plates (0.25 mm) of silica gel 60F-254 (E. Merck, Darmstadt, GFR); zones of colorless compounds were detected by UV light and by spraying the plates with 0.1 M ceric sulfate in 2 M sulfuric acid, with subsequent heating. Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. IR spectra were recorded with a Perkin-Elmer 457 grating spectrophotometer. ¹H and ¹³C NMR spectra were determined by Mr. P. BHATÉ at 200 and 50 MHz, respectively, with a Bruker WP-200 spectrometer. Chemical shifts refer to an internal standard of tetramethylsilane (δ 0.00). Elemental analysis were performed by Atlantic Microlab, Inc., Atlanta, Georgia.

14-*O*-*tert*-Butyldimethylsilyladriamycinone (7)

To a solution of adriamycinone (0.35 g, 0.85 mmol) in DMF (2 ml), imidazole (0.15 g, 2.2 mmol) and *tert*-butylchlorodimethylsilylamine (0.13 g, 0.86 mmol) were added, and the mixture was stirred at 25°C. After 2 hours, the same quantities as before of imidazole and the silylating reagent were added. The mixture was stirred for 1 hour at room temperature and then diluted with CH₂Cl₂ (200 ml), washed with 5% HCl (50 ml) and H₂O (50 ml), and dried over magnesium sulfate containing ~0.5 g of sodium carbonate. The mixture was filtered and the filtrate evaporated. The residue crystallized upon

* Further, comparative tests are in progress and will be reported elsewhere.

addition of absolute EtOH; yield 0.27 g. The mother liquors were evaporated and the residue purified by column chromatography on silica gel (30 g), with 9:1, toluene - acetone as eluant. Fractions containing the product of Rf 0.51 (3:1, toluene - acetone) were pooled and evaporated. The 14-silylated adriamycinone (**7**, 0.1 g) precipitated from an ethereal solution upon addition of hexane; the overall yield was 0.37 g (82.5%), mp 205°C, $[\alpha]_D^{25} + 88.6^\circ$ (*c* 0.04, CHCl₃); IR $\nu_{\text{max}}^{\text{KBr}}$ 3440 (OH), 1735 (CO), 1615, 1580 (H-bonded quinone), 1280 (SiMe) and 830 cm⁻¹ (Si-C); ¹³C NMR δ 211.4 (C-13), 186.9, 186.5 (C-5, 12), 161.1 (C-4), 156.1, 155.6 (C-6, 11), 135.8 (C-2), 135.5, 133.7 (C-6a, 10a, 12a), 120.8 (C-4a), 119.8 (C-1), 118.5 (C-3), 111.5, 111.1 (C-5a, 11a), 77.0 (C-9), 66.8, 62.1 (C-7, 14), 56.7 (OMe), 35.7 (C-8), 33.8 (C-10), 25.9 (Me of Bu^tSi), 18.5 (Si-C of Bu^tSi) and -5.4 (SiMe₂).

Anal Calcd for C₂₇H₃₂O₆Si (528.64): C 61.35, H 6.10.

Found: C 61.15, H 6.12.

14-*O*-*tert*-Butyldimethylsilyl-7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy- α -L-*lyxo*-hexopyranosyl)adriamycinone (**9**)

This compound was prepared by two different procedures.

(a) A mixture of compound **7** (541.2 mg, 1.02 mmol), yellow mercuric oxide (807 mg), mercuric bromide (55 mg) and powdered molecular sieve 4 Å (~2 g) in CH₂Cl₂ (30 ml) was stirred for 0.5 hour at 25°C, and then a solution of the chloride **8**, prepared⁴⁾ from 426 mg (2 mmol) of 3,4-di-*O*-acetyl-L-fucal in CH₂Cl₂, was added. The mixture was stirred overnight at 25°C, diluted with 50 ml of CH₂Cl₂, and filtered through Celite. The filtrate was washed with 10% aqueous potassium iodide (2 × 30 ml) and twice with an excess of H₂O. The organic layer was dried with magnesium sulfate, filtered, and evaporated under diminished pressure, affording a red oil that crystallized from acetone - ethyl ether - hexane; yield 627 mg (82.5%), mp 132~134°C, $[\alpha]_D^{25} + 447^\circ$ (*c* 0.02, CHCl₃); IR $\nu_{\text{max}}^{\text{KBr}}$ 3470 (OH), 1755, 1745, 1740 (CO), 1615 and 1580 (H-bonded quinone), 1270 (SiMe) and 835 cm⁻¹ (Si-C); ¹³C NMR δ 211.1 (C-13), 187.2, 186.7 (C-5, 12), 170.6, 169.9 (CO), 161.1 (C-4), 156.3, 155.8 (C-6, 11), 135.7 (C-2), 134.2 (×2), 133.7 (C-6a, 10a, 12a), 121.0 (C-4a), 119.9 (C-1), 118.5 (C-3), 111.6, 111.5 (C-5a, 11a), 101.2 (C-1'), 77.0 (C-9), 70.1, 69.5, 66.6, 66.4, 65.9 (C-3', 4', 5', 7, 14), 56.7 (OMe), 35.8 (C-8), 34.0 (C-10), 29.8 (C-2'), 25.9 (Me of Bu^tSi), 20.8, 20.7 (AcO), 18.6 (Si-C of Bu^tSi), 16.6 (C-6'), -1.0 and -1.1 (SiMe₂).

Anal Calcd for C₃₇H₄₀O₁₄Si (742.86): C 59.82, H 6.24.

Found: C 59.59, H 6.28.

(b) 7-*O*-(3,4-Di-*O*-acetyl-2,6-dideoxy- α -L-*lyxo*-hexopyranosyl)adriamycinone³⁾ (**4**, 0.44 g, 0.7 mmol) was dissolved in dry DMF (2 ml). To this solution were added imidazole (0.12 g, 1.76 mmol) and *tert*-butylchlorodimethylsilane (0.137 g, 0.91 mmol) and the mixture was stirred for 20 hours at room temperature. TLC monitoring showed some starting material remaining, and so additional silylating reagent (0.06 g, 0.4 mmol) was added. After an additional 2-hour, the starting material had disappeared. The mixture was poured into H₂O (30 ml) and extracted with CH₂Cl₂ (100 ml, twice). The extract was washed with 5% HCl (50 ml), H₂O (50 ml) and 10% aqueous sodium hydrogencarbonate, dried over magnesium sulfate, and evaporated. The residue was dissolved in CH₂Cl₂ (2 ml) and reprecipitated by addition of hexane; yield 0.44 g (84.6%).

14-*O*-*tert*-Butyldimethylsilyl-7-*O*-(2,6-dideoxy- α -L-*lyxo*-hexopyranosyl)adriamycinone (**10**)

Compound **9** (0.40 g, 0.54 mmol) was dissolved in MeOH (30 ml) and the solution was stirred for 1 hour at 25°C with 25% sodium methoxide in MeOH (0.31 ml, 1.35 mmol). The mixture was made neutral by the addition of Dry Ice and evaporated to 15 ml under diminished pressure. The solution was diluted with CH₂Cl₂ (200 ml), extracted with H₂O (50 ml, twice), dried over magnesium sulfate, and evaporated. The residue was dissolved in a small volume of hot MeOH, and the product precipitated upon cooling; yield 0.31 g (88%) of a solid that was sufficiently pure for the next step of the synthesis. Further purification could be achieved by dissolving the solid in the minimal amount of CH₂Cl₂ and addition of ether; Rf 0.2 (1:1 toluene - acetone); mp 227°C, $[\alpha]_D^{25} + 160^\circ$ (*c* 0.02, CHCl₃); IR $\nu_{\text{max}}^{\text{KBr}}$ 3450 (OH), 1735 (CO), 1620, 1585 (H-bonded quinone), 1290 (SiMe) and 835 cm⁻¹ (Si-C); ¹³C NMR δ 211.5 (C-13), 186.4, 186.3 (C-5, 12), 160.7 (C-4), 155.9, 154.4 (C-6, 11), 136.1 (C-2), 135.4, 134.6, 133.8 (C-6a, 10a, 12a), 120.0 (C-4a), 119.7 (C-1), 118.9 (C-3), 110.7, 110.6 (C-5a, 11a), 100.8 (C-1'), 75.2 (C-9), 70.3, 69.4, 66.6, 65.2, 64.7 (C-3', 4', 5', 7, 14), 56.5 (OMe), 32.4 (C-8), 32.0 (C-10),

30.5 (C-2'), 25.6 (Me of Bu^tSi), 18.0 (Si-C of Bu^tSi), 16.8 (C-6') and -5.5 (SiMe).

Anal Calcd for C₃₈H₄₈O₁₂Si (658.78): C 60.17, H 6.43.

Found: C 59.96, H 6.48.

7-O-(2,6-Dideoxy- α -L-lyxo-hexopyranosyl)adriamycinone (11)

Compound **10** (0.20 g, 0.3 mmol) was dissolved in a mixture of CH₂Cl₂ (10 ml), oxolane (20 ml) and pyridine (0.1 ml), and 1 M tetrabutylammonium fluoride (0.45 ml, 0.45 mmol) was added. After 1 hour, the mixture was diluted with 400 ml of CH₂Cl₂ and extracted with 50-ml portions of saturated NaCl, 5% HCl, H₂O and 10% sodium hydrogencarbonate. The extract was dried over magnesium sulfate and evaporated. Compound **11** crystallized from a concentrated solution in CH₂Cl₂; yield 86 mg. Dilution of the mother liquors with ether gave an additional 52 mg; total yield 83%; mp 175°C, [α]_D²⁵ +176° (c 0.02, CHCl₃); Rf 0.26 (2: 1, acetone - toluene); IR $\nu_{\text{max}}^{\text{KBr}}$ 3450 (br, OH), 1725 (CO), 1620 and 1580 cm⁻¹ (H-bonded quinone).

Anal Calcd for C₂₇H₂₃O₁₂·0.5H₂O (553.53): C 58.59, H 5.28.

Found: C 58.38, H 5.12.

Acknowledgments

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References

- 1) FUCHS, E.-F.; D. HORTON, W. WECKERLE & E. WINTER-MIHALY: Synthesis and antitumor activity of sugar-ring hydroxyl analogues of daunorubicin. *J. Med. Chem.* 22: 406~411, 1979
- 2) HORTON, D. & W. PRIEBE: Glycon- and C-14-modified, antitumor doxorubicin analogs. *In Anthracycline Antibiotics. Ed., H. S. EL KHADEM*, pp. 197~224, Academic Press, Inc., New York, 1982 and references cited therein.
- 3) HORTON, D.; W. PRIEBE & W. R. TURNER: Synthesis and antitumor activity of 7-O-(3,4-di-O-acetyl-2,6-dideoxy- α -L-lyxo-hexopyranosyl)adriamycinone. *Carbohydr. Res.* 94: 11~25, 1981
- 4) EL KHADEM, H. S.; D. L. SWARTZ, J. K. NELSON & L. A. BERRY: Synthesis of 2,6-dideoxy- α -L-lyxo- and 2,6-dideoxy- α -L-arabino-hexopyranosyl halides. *Carbohydr. Res.* 58: 230~234, 1977
- 5) HORTON, D. & W. PRIEBE: 14-Esters of 7-O-(3,4-di-O-acetyl-2,6-dideoxy- α -L-lyxo-hexopyranosyl)adriamycinone: Synthesis and antitumor activity. *J. Antibiotics* 36: 1211~1215, 1983