Communications to the editor

STEREOCHEMICAL REQUIREMENTS IN THE ANTITUMOR ANTHRACYCLINES

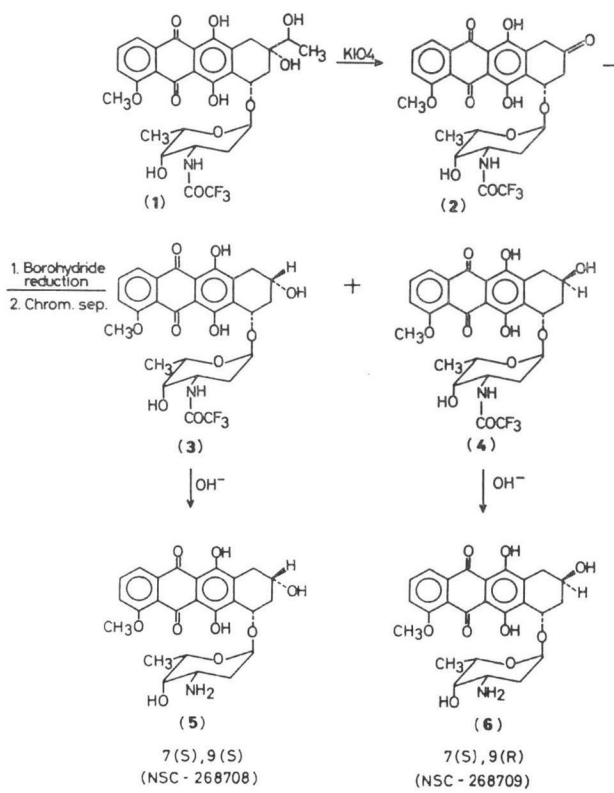
Sir:

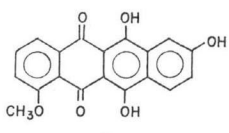
We wish to report the synthesis, DNA binding data and early biological evaluation of 9-deacetyldaunorubicin [NSC 268708, (**5**)] and the corresponding C-9 epimer [NSC 268709, (**6**)]. Our findings are interesting as regards to the dependence of the DNA-binding properties upon the stereochemistry at C-9 asymmetric center and the relationships of these properties with antitumor efficacy.

Synthesis of **5** and **6** was performed starting from 13-dihydro-N-trifluoroacetyldaunorubicin (**1**), obtained in quantitative yield by N-trifluoroacetylation of 13-dihydrodaunorubicin[1] with trifluoroacetic anhydride in chloroform followed by hydrolysis of the O-trifluoroacetyl groups with methanol. Compound **1** was converted by treatment with an excess of sodium metaperiodate in a mixture of *t*-butyl alcohol and water (1:1 by vol.) at room temperature to the less polar (Rf 0.57, TLC-I)* ketone **2**, which appeared insoluble in the reaction mixture (65% yield). Compound **2**, dark red solid, m.p. 200°C (dec.), anal. calcd.: for $C_{27}H_{24}F_3NO_{10}$, C 55.95, H 4.18; found C 55.65, H 4.26, displayed the same ultraviolet and visible spectrum as **1** and by refluxing with 0.1 N HCl in aqueous dimethoxyethane for 2 hours was quantitatively degraded to fully aromatized **7**, *m/e* 336 (M^+); *m/e* 318 ($M-H_2O$); *m/e* 290 ($M-H_2O-CO$); λ_{max} ($CHCl_3$) 475, 505, 542 nm.

Compound **2** was reduced with $NaCNBH_3$ in aqueous dioxane to give the mixture of the N-trifluoroacetates **3** and **4** in approximately the ratio 9:1. During the reaction the pH of the mixture was kept in the range 3~4 by addition of 1 N aqueous HCl. Separation of the components by chromatography on a column of silicic acid using chloroform - acetone (4:1 by vol.) as eluting agent, gave compound **3**, m.p. 206°C (dec.), Rf 0.36 (TLC-I), anal.: calcd. for $C_{27}H_{26}F_3NO_{10}$, C 55.76, H 4.52; found C 55.98, H 4.71; and **4**, m.p. 180~182°C, Rf 0.23 (TLC-I), anal.: found C 55.54, H 4.77. Hydrolysis of **3** and **4** with 0.1 N aqueous NaOH for 30 minutes gave

Fig. 1.





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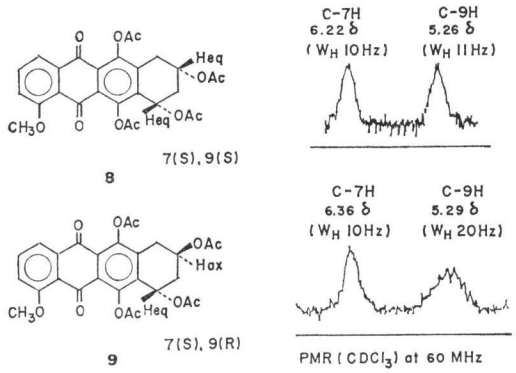
* For homogeneity and identity test thin-layer chromatography (Merck F 254) with solvent system chloroform - acetone (2:1 by vol.) (TLC-I) and with solvent system chloroform - methanol - water (13:6:1 by vol.) (TLC-II) were used.

Fig. 2. Assignment of stereochemistry to diastereomeric 9-deacetyldaunomycinone acetates



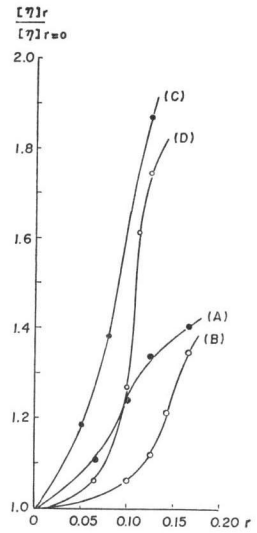
the corresponding aminoglycosides **5** and **6**, which were isolated as hydrochlorides. Compound **5**, m.p. 166~167°C; Rf 0.55 (TLC-II); anal.: calcd. for $C_{25}H_{27}NO_9 \cdot HCl$, C 57.52, H 5.42, N 2.68; found C 57.16, H 5.45, N 2.42; and **6**, m.p. 173~175°C; Rf 0.40 (TLC-II); anal.: found C 57.61, H 5.52, N 2.63; displayed the same UV and visible spectra as the starting compound, indicating the integrity of the chromophoric system during the above reactions sequence and the absence of non-conjugated carbonyl group (1700~1730 cm^{-1}) in the IR spectrum.

Table 1. Binding parameters with native calf-thymus DNA*

Compound	$K_{app} \cdot 10^{-5}$	n
Daunorubicin	4.5	0.16
Doxorubicin	3.7	0.18
9-Deacetyldaunorubicin (**5**)	2.2	0.14
9-Epi-deacetyldaunorubicin (**6**)	0.8	0.16

* For experimental details see ref. 3.

Fig. 3. Ratios of intrinsic viscosity of:
(A) 9-Deacetyl-daunorubicin (**5**)
(B) 9-Epi-9-deacetyl-daunorubicin (**6**)
(C) Daunorubicin and (D) doxorubicin-native DNA complexes to that of native DNA plotted against r (moles of antibiotic/DNA-P). For experimental details see ref. 3



Assignment of the stereochemistry at C-9 in **5** and **6** was based on the study of the pmr spectra of tetraacetyl derivatives **8** and **9**, obtained respectively from **5** and **6** upon hydrolysis with 0.2 N aqueous HCl at 70°C followed by acetylation (Ac_2O, pyridine) of the resulting water insoluble aglycones. The pmr spectrum (60 MHz, $CDCl_3$) of **8**, *m/e* 524 (M^+); *m/e* 482 ($M-CH_2CO$); *m/e* 440 ($M-2CH_2CO$); *m/e* 320 ($M-2CH_2CO-2CH_3COOH$) showed signals at δ 1.98 and 2.04 (two s, CH_3COO at C-7 and C-9), δ 2.42 and 2.48 (two s, CH_3COO at C-6 and C-11), δ 3.96 (s, CH_3O), δ 5.26 (m, W_H 11 Hz,

Table 2. Activity of diastereoisomeric 9-deacetyldaunorubicin on P 388 lymphocytic leukemia in CDF_1 mice

Treatment ip on days 1 to 9. Median survival time expressed as per cent of controls (T/C%).[a]

Compound	Selected doses (mg/kg/day)				
	1.56	3.13	6.25	12.5	25.0
9-Deacetyldaunorubicin (**5**)	155	174	180	228	73
9-Epi-deacetyldaunorubicin (**6**)	142	157	171	66	66

[a] Data obtained under the auspices of NCI. Screener: A.D. Little Inc. In the same experiment optimal dose of daunorubicin was 1.00 mg/kg (T/C%=171) and of adriamycin was 2.00 mg/kg (T/C%=180). For experimental details see ref. 5.

C-9H), δ 6.22 (m, W_H 10 Hz, C-7H), δ 7.2~8.0 (m, 3 aromatic protons). Similarly the spectrum of **9**, *m/e* 482 (M^+), showed signals at δ 2.00 and 2.06 (two s, CH_3COO at C-7 and C-9), δ 2.41 and 2.46 (two s, CH_3COO at C-6 and C-11), δ 3.93 (s, CH_3O), δ 5.29 (m, W_H 20 Hz, C-9H), δ 6.36 (m, W_H 10 Hz, C-7H), δ 7.05~7.85 (m, 3 aromatic protons). Major diagnostic difference in the pmr spectra of the two epimers is presented in Fig. 2, showing that the width of C-7 H multiplet is the same in **8** and **9** whereas that of C-9 H is larger in **9** indicating a quasi-axial orientation.

As the main biochemical effects of anthracycline antibiotics are concerned with nucleic acid synthesis and the binding of these drugs to DNA is considered responsible for their interference with template DNA function[2], the interaction of **5** and **6** with native DNA was studied by equilibrium dialysis (Table 1) and low shear viscosimetry (Fig. 3). The results were compared with those obtained for daunorubicin and doxorubicin.[3] The K_{app} values of both deacetyl derivatives are lower than those of daunorubicin and doxorubicin. In particular, the K_{app} value of **6** indicates that in this derivative the beta orientation of C-9 OH group is less favourable to its interaction with the first DNA phosphate away from the intercalation site.[4] These results are confirmed by those obtained by low shear viscosimetry (Fig. 3) which show a similar trend.

Preliminary activity data of **5** and **6** are reported in Table 2. The two epimers showed a different efficacy as antitumor agents in the laboratory animals, the 9(S) compound showing a higher selectivity of inhibition of tumor development in comparison with the toxic effects in the host. Our results indicate that in the antitumor anthracyclines stereochemistry at the C-9 center is an important molecular requirement for optimal pharmacological activity. It is also worth noting that in the C-9 deacetyl derivatives described here, the effect of stereochemistry on pharmacological activity parallels that on DNA complexing ability.

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References

1) JOLLES, G. & G. PONSINET: Antibiotic 20,798 RP and antibiotic 27,706RP. German Offenlegungsschrift 2202690, July 27, 1972
2) DI MARCO, A. & F. ARCAMONE: DNA-complexing antibiotics: Daunomycin, adriamycin, and their derivatives. Arzneim. Forsch. 25: 368~375, 1975 and references therein.
3) ARLANDINI, E.; A. VIGEVANI & F. ARCAMONE: Interaction of new derivatives of daunorubicin and adriamycin with DNA. Il Farmaco Ed. Sci. (Pavia) 32: 315~323, 1977
4) PIGRAM, W. J.; W. FULLER & L. D. HAMILTON: Stereochemistry of intercalation: Interaction of daunomycin with DNA. Nature, New Biology 235: 17~19, 1972
5) GERAN, R. L.; N. H. GREENBERG, M. M. MACDONALD, A. M. SCHUMACHER & B. J. ABBOT: Cancer Chemother. Rep. Part 3, 3: 9, 1972