SYNTHESIS OF 10-METHOXYDAUNORUBICINS AND OF 10(R)-METHOXYDOXORUBICIN VIA OPENING OF AN OXIRANE INTERMEDIATE

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Abstract - Epoxidation of 9,10-anhydro-13-dihydro-N-trifluoroacetyldaunorubicin provides stereoselectively the corresponding α oxirane derivative. Opening of the intermediate by methanol affords 10(R)- and 10(S)-methoxydaunorubicins. From ^{1}H n.m.r. studies it appears that the conformation of ring A of 10(S)-derivative has changed to half-boat. The preliminary biological data of these analogs, as well as of 10(R)-methoxydoxorubicin, are reported.

Although chemical modifications of the antitumor anthracyclines daunorubicin and doxorubicin and related compounds have been extensively carried out in this as well as in other laboratories, the only reported compound possessing oxirane ring on the alicyclic portion of the tetracyclic aglycone moiety is totally synthetic 9,10-epoxy-10-deoxy-γ-rhodomycinone described by A. S. Kende et al.[1]. We have therefore investigated the 9,10-epoxides of daunorubicin in order to evaluate their usefulness for the semisynthesis of C-10 substituted analogs. In fact, other biosynthetic anthracycline glycosides[2] (rhodomycin A and B, cytromycin) bear substituents at the C-10 benzylic centre whereas analogs of daunorubicin and doxorubicin, modified at the above mentioned centre, have not yet been described.

9,10-Anhydro-N-trifluoroacetyldaunorubicin (1), which is easily obtained by treatment of daunorubicin hydrochloride with trifluoroacetic anhydride in the presence of an organic base[3], was an useful starting material for the preparation of a C-9, C-10 epoxide. In order to perform the epoxidation reaction it was necessary to reduce the carbonyl group of the side-chain to the corresponding alcohol 2. In fact the epoxidation in alkaline medium, a well-known procedure[4] required for α, β-unsaturated ketones, promptly caused the destruction of the substrate. The reduction was selectively performed using sodium cyanoborohydride in aqueous methanolic solution at pH below 4 and the formation of the oxirane ring was achieved by treatment with m-chloroperbenzoic acid giving 3 in quantitative yield. Regeneration of the side-chain ketone function with contemporary oxidation of the hydroxyl group in the sugar moiety, as confirmed by the molecular peak (m/e 619 M^{+}) in the field desorption mass spectrum of 4, was obtained upon oxidation with dimethyl sulfoxide -dicyclohexylcarbodiimide, using pyridinium trifluoroacetate as catalyst[5]. The ^{1}H n.m.r. spectrum of 4 showed the presence of equal amounts of two isomers : the chiral centre responsible for the formation of this epimeric mixture was present in the sugar moiety; in fact by treatment with

gaseous hydrogen chloride in acetic acid 4 underwent the loss of the sugar residue giving the chloridrine 5 (m/e 432 M^+), which by mild treatment gave easily the aglycone 6, as single isomer. The ^1H n.m.r. spectrum of 6 showed a long range coupling constant (J = 0.6 Hz) between the equatorial proton at C-8 and that at C-10, which must be consequently equatorial, a known property of daunomycinone derivatives[6]. Moreover the J_{vic} values among the protons at C-7 and C-8 ($J_{H-7, H-8_{ax}}$ = 4.3 Hz; $J_{H-7, H-8_{eq}}$ = 1.5 Hz) were similar to those of daunomycinone, indicating that the conformation of the alicyclic ring was unchanged. These data are in agreement with the α configuration of the oxirane ring in compound 6.

Opening of the epoxide allowed the introduction of substituents at C-10. The treatment of 6 with methanol in the presence of a catalytic amount of p-toluenesulfonic acid gave a mixture of two aglycones 7 and 8 approximately in the ratio 4:1, both of which have a methoxyl group at C-10 and different stereochemistry at this centre. The major compound 7 (m/e 428 M^+) gave 7,9-isopropylidene derivative 9 by treatment with dimethoxypropane and catalytic amount of p-toluenesulfonic acid, thus showing that the hydroxyl groups at C-7 and C-9 had cis relationship. On the other hand, the signal of C-10 H in ^1H n.m.r. spectrum appeared as doublet at 4.67 δ with a long range coupling constant with C-8 Heq of about 1 Hz, indicating the equatorial orientation of the former. On the contrary the ^1H n.m.r. spectrum of 8 showed the C-10 H signal as a sharp singlet indicating that this compound is the isomer in which H-10 has an axial orientation. Both 7 and 8 afforded 7-deoxydaunomycinone[6] (10) by catalytic hydrogenolysis of the benzylic groups, thus confirming unequivocally the structure attributed to epoxide 6, and to 7 and 8 derived from trans and cis opening of the oxirane ring, respectively[7]. It is to be pointed out that in 8 the J_{vic} of C-7-H are both 6 Hz. These values, which are different from those of daunomycinone and of compound 7 (in the range 1-2 and 4-5 Hz for the usual half-chair conformation), correspond to boat conformation (Fig. 1 shows H-7 signals of 7 and 8 after D_2O exchange).

The new anthracyclinones 7 and 8 were coupled with 1-chloro-N,O-trifluoroacetyldaunosamine[8], using silver triflate as catalyst[9], to give the corresponding glycosides 11 and 12, which after hydrolysis of the N-protecting group afforded 10(R)-methoxydaunorubicin 13 and 10(S)-methoxydaunorubicin 14. The corresponding doxorubicin analog 15 was obtained from 13 by treatment with bromine to give the 14-bromo derivative followed by reaction with an aqueous solution of sodium formate.

The new glycosides underwent biological testing under the auspices of the National Cancer Institute. The preliminary data are reported in the Table.

Table

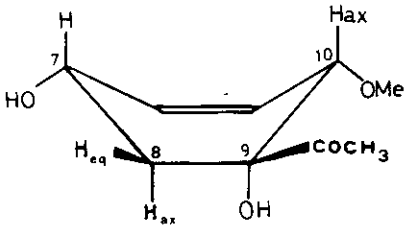
Activity on P 388 lymphocytic leukemia in mice[a] (NCI data, screener A.D. Little).

	OD[b]	AST[c]
13	12.5	133
14	50.0	no activity
15	6.25	141

a) i.p. treatment, schedule q 4 d 5, 9, 13.
b) optimal dose(mg/kg).
c) average survival time as % of controls.

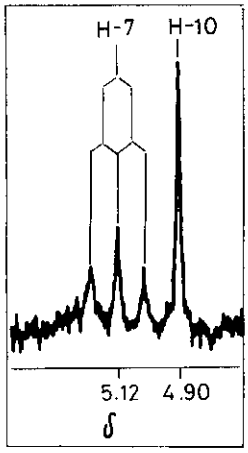
The two epimers show different pharmacological properties : in fact 13, showing the same absolute configuration at C-10 as the known biosynthetic anthracycline, exhibits distinct increase of survival time of treated animals in respect to controls : on the contrary the 10(S)-methoxy analogue 14 is inactive at the maximum dose tested (50 mg/kg). These results indicate that the antitumor activity is deeply affected by modifications which lead to changes of the conformation of ring A as well as of stereochemistry of the substituent at C-10.

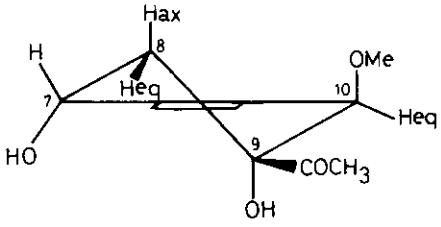
Figure 1



8

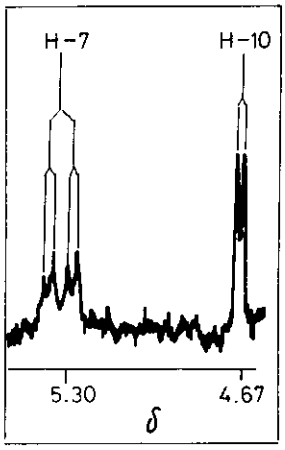
$J_{H-7H-8ax}$ = 6 Hz
$J_{H-7H-8eq}$ = 6 Hz

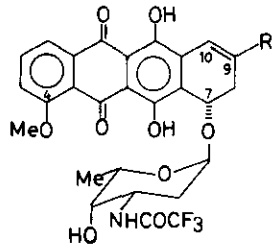




7

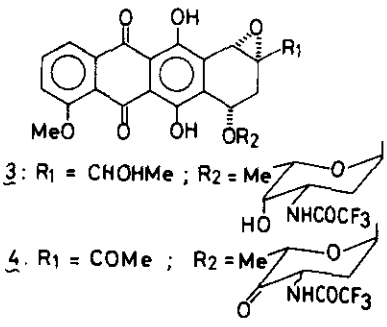
$J_{H-8eqH-10eq}$ = 1 Hz
$J_{H-7H-8ax}$ = 5 Hz
$J_{H-7H-8eq}$ = 2 Hz





1. R = COMe

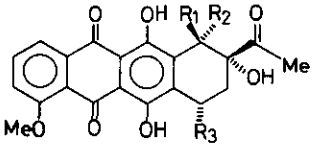
2: R = CHOHMe



3: R_1 = CHOHMe ; R_2 = Me

4. R_1 = COMe ; R_2 = Me

6 R_1 = COMe ; R_2 = H

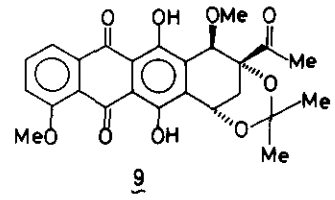


5. R_1 = Cl , R_2 = H ; R_3 = OH

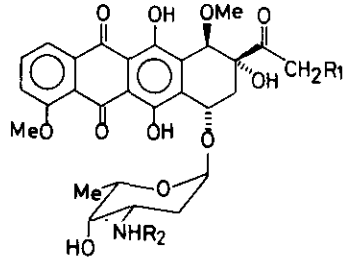
7: R_1 = OMe ; R_2 = H ; R_3 = OH

8: R_1 = H ; R_2 = OMe ; R_3 = OH

10. R_1 = R_2 = R_3 = H



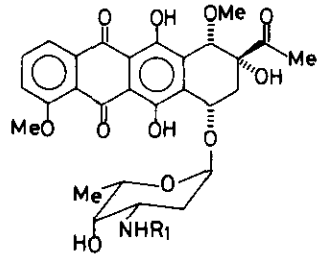
9



11. R_1 = H ; R_2 = $COCF_3$

13: R_1 = R_2 = H

15: R_1 = OH ; R_2 = H



12: R_1 = $COCF_3$

14 R_1 = H

EXPERIMENTAL

Melting points were determined in capillary tubes and are uncorrected. U.V. spectra were measured on a Hitachi ESP-3T spectrophotometer. ^{1}H n.m.r. spectra were measured in $CDCl_3$ solution on Bruker HX 90 or Varian A-60/A or EM 360 spectrometers. Mass spectra were recorded on a Varian-Mat 311 A spectrometer.

9,10-Anhydro-13-dihydro-N-trifluoroacetyldaunorubicin (2) - A solution of 9,10-anhydro-N-trifluoroacetyldaunorubicin (1) (6 g, $10 \cdot 10^{-3}$ mol) in methanol (2 l) and 0.1 N aqueous hydrogen chloride (50 ml) was treated with $NaCNBH_3$ (4.0 g, $63 \cdot 10^{-3}$ mol) in water (200 ml) for 48 h at room temperature, keeping the pH below 4. The residue, obtained after neutralization of reaction mixture and evaporation of solvent, was purified by chromatography on column of silicic acid using as eluent the system $CHCl_3$: $(CH_3)_2CO$ (95 : 5 v/v) giving 5 g of pure 2, m.p. 165° (dec.), λ max ($CHCl_3$) 520, 556 nm, m/e 607 (M^+).

9-Deoxy-9,10-epoxy-13-dihydro-N-trifluoroacetyldaunorubicin (3) - A solution of 2 (2 g, $3.2 \cdot 10^{-3}$ mol) in chloroform (400 ml) was treated with m-chloroperbenzoic acid (1 g, $6 \cdot 10^{-3}$ mol) and warmed at 80° for 3 h. The initial cherry colour of solution gradually changed to red. The residue 3 (2 g), obtained after neutralization of reaction mixture and evaporation of solvent, showed λ max ($CHCl_3$) : 490, 504, and 540 nm in agreement with the disappearance of double bond at C-9, C-10 of 2, m/e 623 (M^+).

9-Deoxy-9,10-epoxy-daunomycinone (6) - To a stirred solution of 3 (3.85 g, $6 \cdot 10^{-3}$ mol) in anhydrous dimethyl sulfoxide (100 ml) under nitrogen dicyclohexylcarbodiimide (3.8 g, $18 \cdot 10^{-3}$ mol), anhydrous pyridine (0.5 ml, $6 \cdot 10^{-3}$ mol) and trifluoroacetic acid (0.23 ml, $3 \cdot 10^{-3}$ mol) were successively added. The reaction mixture was stirred at room temperature for 15 h, then diluted with chloroform (500 ml). The residue obtained by evaporation of solvent was taken up in ethyl acetate, the insoluble dicyclohexylurea filtered off and the solution evaporated to dryness to give compound 4 in quantitative yield, m/e 619 (M^+), ^{1}H n.m.r. : 1.33 and 1.42 δ (two d, CH_3-C-5'), 2.24 δ (s, CH_3CO), 4.03 δ (s, CH_3O), 4.7 δ (m, C-10-H), 5.2-5.6 δ (m, C-7-H and C-1'-H), and 13.38, 13.82, and 13.83 δ (s, phenolic protons).

Then a solution of 4 (2 g, $3 \cdot 10^{-3}$ mol) in benzene (270 ml) was treated with acetic acid containing gaseous hydrogen chloride. The reaction mixture, after 1 h at room temperature, was diluted with water and extracted with chloroform, giving, after evaporation of solvent, the chloridrine 5 in quantitative yield, m/e 432 (M^+) in field desorption mass spectrum. The unstable 5 was promptly converted to desired epoxide 6 by treatment of its chloroformic solution with silica gel buffered to pH 7 with phosphate buffer, m/e 396 (M^+), ^{1}H n.m.r. : 2.28 δ (s, CH_3CO), 2.48 δ (dd, J' = 4.3; J" = 16.5 Hz, C-8-H_{ax}), 2.70 δ (d, J = 11.2 Hz, C-7-OH), 2.68 δ (ddd, J' = 1.5, J" = 16.5, J''' = 0.6 Hz, C-8-H_{eq}), 4.10 δ (s, CH_3O), 4.90 δ (d, J = 0.6 Hz, C-10-H), 5.35 δ (ddd, J' = 1.5, J" = 4.3, J''' = 11.2 Hz, C-7-H), and 13.45 and 13.70 δ (s, phenolic protons).

10(R)-Methoxydaunomycinone (7) and 10(S)-Methoxydaunomycinone (8) - To a solution of 6 (1 g, $2.5 \cdot 10^{-3}$ mol) in anhydrous methanol (100 ml) a catalytic amount of p-toluenesulfonic acid was added

and after 1 h at 50° the reaction mixture was diluted with chloroform, neutralized and evaporated to dryness. The residue, a mixture of 7 and 8 in the ratio 4 : 1 approximately, was chromatographed on column of silicic acid using the mixture of ethyl acetate–toluene–petroleum ether (3:2:2 v/v) as eluting agent. Pure 7 (0.45 g) and 8 (0.12 g) were obtained.

7 – m.p. 220° (dec.), $[\alpha]_D^{20}$ = +206° (c 0.1, $CHCl_3$), m/e 428 (M^+), 396 (M–CH_3OH), 353 (M–CH_3OH–CH_3CO), ^1H n.m.r. : 2.45 δ (s, CH_3CO), 3.51 δ (s, C–10–OCH_3), 3.64 δ (d, J = 6.5 Hz, C–7–OH), 4.08 δ (s, C–4–OCH_3), 4.67 δ (d, J = 1.0 Hz, C–10–H), 5.30 δ (ddd, J' = 6.5, J" = 5.0, J''' = 2.0 Hz, C–7–H), and 13.68 and 14.07 δ (s, phenolic protons).

8 – m.p. 156° (dec.), m/e 428 (M^+), $[\alpha]_D^{20}$ = +106° (c 0.05, $CHCl_3$), ^1H n.m.r. : 2.28 δ (s, CH_3CO), 3.64 δ (s, C–10–OCH_3), 4.07 δ (s, C–4–OCH_3), 4.23 δ (d, J = 6.0 Hz, C–7–OH), 4.90 δ (s, C–10–H), 5.12 δ (ddd, J' = J" = J''' = 6.0 Hz, C–7–H), and 13.80 and 14.21 δ (s, phenolic protons).

7,9-Isopropylidene-10(R)-methoxydaunomycinone (9) – A solution of 7 (0.1 g, $2.5 \cdot 10^{-4}$ mol) in anhydrous dioxane (10 ml) was treated with an excess of 2,2-dimethoxypropane (30 ml) in presence of a catalytic amount of p-toluenesulfonic acid. After 48 h at 50° the reaction mixture was diluted with chloroform (200 ml), neutralized and evaporated to dryness. The residue, chromatographed on column of silicic acid using the mixture $CHCl_3$: $(CH_3)_2CO$ (95 : 5 v/v) as eluting agent, afforded pure 9 : m/e 468 (M^+), 410 (M–$(CH_3)_2CO$), 378 (M–$(CH_3)_2CO$–CH_3OH), ^1H n.m.r. : 1.20 and 1.47 δ (two s, geminal CH_3 groups), 2.48 δ (s, CH_3CO), 3.58 δ (s, C–10–OCH_3), 4.09 δ (s, C–4–OCH_3), 4.75 δ broad s, W_H = 2 Hz, C–10–H), 5.47 δ (broad s, W_H = 6 Hz, C–7–H), and 13.42 and 13.68 δ (s, phenolic protons).

7-Deoxydaunomycinone (10) – A solution of 7 or of 8 (0.2 g) in dioxane (30 ml) was hydrogenated in presence of 5 % Pd/$BaSO_4$ (0.2 g) as catalyst, giving 7-deoxydaunomycinone identical with a synthetic specimen prepared from daunomycinone[6].

10(R)-Methoxydaunorubicin hydrochloride (13) – To a solution of 7 (0.43 g, $1 \cdot 10^{-3}$ mol) in anhydrous methylene chloride (200 ml) 1-chloro-N,O-trifluoroacetyldaunosamine (0.43 g, $1.2 \cdot 10^{-3}$ mol) was added[8]. Then $AgSO_3CF_3$ (0.32 g, $1.2 \cdot 10^{-3}$ mol) dissolved in anhydrous diethyl ether (26 ml) was added to the solution at room temperature over 10 min; finally, anhydrous collidine (0.2 ml, $1.4 \cdot 10^{-3}$ mol) was added to the reaction mixture. After 40 min, the mixture was treated with saturated aqueous solution of $NaHCO_3$ and the organic phase evaporated to dryness. The residue was dissolved in methanol (100 ml) and kept at room temperature for 5 h. The residue, resulted by removal of solvent, was chromatographed on column of silicic acid using the mixture $CHCl_3$: $(CH_3)_2CO$ (4 : 1 v/v) as eluting agent. In addition to unreacted 7, pure 10(R)-methoxy-N-trifluoroacetyldaunorubicin (11) (0.26 g) was obtained : m.p. 190° (dec.) ^1H n.m.r. : 1.30 (d, CH–CH_3); 3.52 δ (s, C–10–OCH_3); 5.30 (m, C–7–H) and 5.53 δ (m, W_H = 7 Hz, C–1'–H_{ax}).

Compound 11 (0.26 g) was dissolved in 0.1 N aqueous sodium hydroxide (50 ml) and after 30 min at 0° the solution was adjusted at pH 8.6 and repeatedly extracted with chloroform. The combined extracts, concentrated to a small volume and acidified at pH 4.5 with 0.1 N methanolic hydrogen chloride, afforded by crystallization 10(R)-methoxydaunorubicin (13) as hydrochloride : m. p. 159°

(dec.); $[\alpha]_D^{20}$ = +316° (c 0.05, MeOH). (Found : C, 55.44; H, 5.47; $C_{28}H_{31}O_{11}N \cdot HCl$ requires C, 56.61; H, 5.44).

10(S)-Methoxydaunorubicin hydrochloride (14) - The coupling reaction between the anthracyclinone 8 (1.1 g, $2.5 \cdot 10^{-3}$ mol) and 1-chloro-N,O-trifluoroacetyldaunosamine (1.1 g, $3 \cdot 10^{-3}$ mol) according to procedure described for 12 afforded 10(S)-methoxydaunorubicin (14) : m. p. 140° (dec.); $[\alpha]_D^{20}$ = = +252° (c 0.05, MeOH). (Found : C, 56.41; H, 5.59; $C_{28}H_{31}O_{11}N \cdot HCl$ requires C, 56.61; H, 5.44).

10(R)-Methoxydoxorubicin hydrochloride (15)- A solution of 10(R)-methoxydaunorubicin hydrochloride (13) (0.78 g, $1.3 \cdot 10^{-3}$ mol) in anhydrous methanol (13.5 ml) and anhydrous dioxane (38 ml), was treated with ethyl orthoformate (1 ml) and with 28.8 ml of a solution of bromine in chloroform (9.5 g of Br_2 to 100 ml with chloroform) at 20° for 60 min. The crude material, obtained by precipitation of the reaction mixture with diethyl ether, was dissolved in acetone (32 ml) and treated with 0.25 N aqueous hydrobromic acid (32 ml). The solution, kept at 30° overnight, was then treated with HCOONa (15 g) dissolved in water (25 ml). After 24 hours at 30° the reaction mixture was extracted with chloroform in order to eliminate the aglycone. The aqueous solution, diluted with methanol (500 ml), was adjusted to pH 7 with $NaHCO_3$ and extracted repeatedly with chloroform. The combined organic extracts were evaporated to a small volume (50 ml) and acidified at 0° to pH 4.5 with 0.1 N methanolic hydrogen chloride. The addition of diethyl ether (1000 ml) afforded crude 10(R)-methoxydoxorubicin hydrochloride (15), which was purified by chromatography on column of cellulose using the mixture chloroform-methanol-water (300 : 55 : 6 v/v) as eluting agent : m.p. 195° (dec.). (Found C, 54.99; H, 5.30; $C_{28}H_{31}O_{12}N \cdot HCl$ requires C, 55.12; H, 5.30).

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