SYNTHESIS AND BIOLOGICAL ACTIVITY OF THE TWO ISOMERIC 2′-*C*-METHYL DAUNOMYCINS

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The title new daunorubicin analogues have been prepared from methyl 4,6-*O*-benzylidene-2-*C*-methyl-α-D-*ribo*-hexopyranosid-3-ulose and daunomycinone. The antitumor activity of these compounds was very similar to that of daunorubicin.

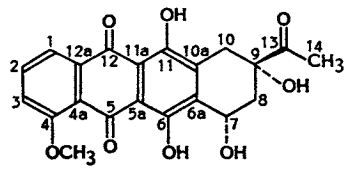
The antibiotic daunorubicin (**1**) is a clinically useful antineoplastic agent.[1] As part of a program directed toward the synthesis of analogues of **1**,[2,3] modified in the amino-sugar moiety, we report now the synthesis of (2′*R*)-2′-*C*-methyl (**17**) and (2′*S*)-2′-*C*-methyl daunomycin (**18**). In view of the excellent antitumor activity of 3′-*C*-methyl daunomycin (**2**)[3] the synthesis of **17** was undertaken in order to explore the biological properties of another daunorubicin analogue in which the C-3′ amino group would be more hindered than in the natural product. The formation of **18** is unexpected.

Chemistry

The known methyl 4,6-*O*-benzylidene-2-*C*-methyl-α-D-*ribo*-hexopyranosid-3-ulose (**4**)[4] was converted to its oxime **5** and the latter was reduced with sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al) to the D-*allo* amine **6**. Protection of the amine by *N*-trifluoroacetylation gave **7** and *N*-bromosuccinimide induced opening of the 4,6-*O*-benzylidene acetal system furnished **8**. Dehydrobromination of **8** in the presence of silver fluoride afforded the unsaturated compound **9**. The double bond of **9** was stereospecifically reduced by hydrogenation to give the β-L-*talo* derivative **10**. Evidence for the L-*talo* configuration of **10** was furnished by its ^{1}H NMR spectrum which revealed at 5.30 ppm a narrow triplet type signal for 4-H. The small ($^3J_{4,5}$=4 Hz) coupling constant between 4-H and 5-H of **10** was indicative of a *cis*-relationship between these hydrogen atoms.

Methyl 4-*O*-benzoyl-2-*C*-methyl-2,3,6-trideoxy-3-trifluoroacetamido-β-L-*talo*-hexopyranoside (**10**) was transformed in two steps, by treatment with aqueous acetic followed by acetylation, into an anomeric mixture of 1-*O*-acetyl-4-*O*-benzoyl-2-*C*-methyl-2,3,6-trideoxy-3-trifluoroacetamido-L-*talo*-hexopyranosides (**11**). This mixture was used, without further purification, for the glycosidation reaction. Glycosylation of daunomycinone (**3**) by **11** was accomplished in 30% yield, with anhydrous *p*-toluene sulfonic acid (pTSA). The reaction furnished three isomeric glycosylation products. These compounds, **12**, **13** and **14** were separated by preparative TLC and their structure established by NMR spectroscopy.

Inspection of the mass and ^{13}C NMR spectra of the glycosylation products revealed that in addition to the expected product **12** two other isomeric compounds **13** and **14** were formed during the reaction. The sugar configuration of **13** was determined to be L-*galacto*. The partial C-2 methyl isomerization, a result of the 1,3-diaxial interaction between the C-2 and C-4 substituents of **11**, has taken place during the reaction which liberated the anomeric hydroxy group. The configuration of the



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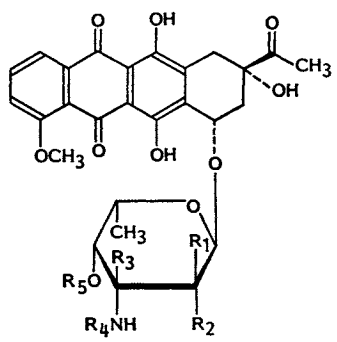
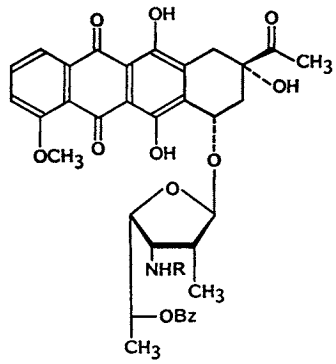
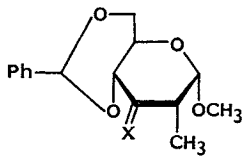


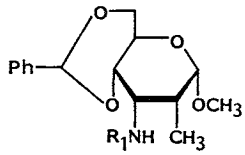


1 $R_1=R_2=R_3=R_4=R_5=H$
2 $R_1=R_2=R_4=R_5=H$ $R_3=CH_3$
12 $R_1=R_3=H$ $R_2=CH_3$ $R_4=COCF_3$ $R_5=COPh$
13 $R_2=R_3=H$ $R_1=CH_3$ $R_4=COCF_3$ $R_5=COPh$
15 $R_1=R_3=R_5=H$ $R_2=CH_3$ $R_4=COCF_3$
16 $R_2=R_3=R_5=H$ $R_1=CH_3$ $R_4=COCF_3$
17 $R_1=R_3=R_4=R_5=H$ $R_2=CH_3\cdot HCl$
18 $R_2=R_3=R_4=R_5=H$ $R_1=CH_3\cdot HCl$

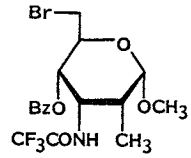
14 $R=COCF_3$



4 X=O
5 X=NOH



6 $R_1=H$
7 $R_1=COCF_3$



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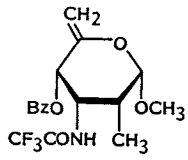

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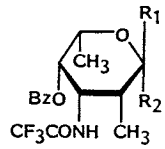

10 $R_1=H$ $R_2=OCH_3$
11 $R_1=H$ or OAc $R_2=OAc$ or H

carbohydrate constituent of **14** was L-*talo*, as in the starting material, however, the sugar was present in the furanose form.

The ^{13}C and ^{1}H NMR spectrum of the three glycosylation products **12**, **13** and **14** revealed structurally diagnostic signals. The ^{13}C NMR spectrum of **12** and **13** appeared characteristic of daunomycinone 7α-glycosides. These compounds were differentiated on the basis of ^{1}H NMR double

resonance experiments. The anomeric proton of both compounds showed a broad singlet indicating the nature of the glycosidic linkage. However, upon irradiation of the C-2′-methyl group signal large and small $^3J_{2,3}$ proton coupling constants could be revealed, respectively, in the spectrum of **12** and **13**. In view of the axially disposed C-4′ substituent only small ^{13}C NMR chemical shift differences were expected in the spectrum of **12** and **13** for C-2′ and C-4′. The ^{13}C NMR spectrum of **14** revealed a typical furanose signal at 87.2 ppm for C-4′ and the ^1H NMR spectrum of **14** showed a characteristic downfield 5′-H signal at 5.35 ppm.

Elimination of the base sensitive C-3′ and C-4′ protecting groups of **12** and **13** was found to proceed more efficiently in two steps rather than in a single step. As the free amines proved to be slightly unstable, their hydrochlorides **17** and **18** were prepared for the biological experiments. Studies on the furanoside **14** were not continued further.

Biological Properties

The cytostatic activity of both 2′-*C*-methyl daunomycin hydrochlorides **17** and **18** against P388 leukemia cells *in vitro* was approximately identical with that of daunorubicin (**1**).

Experimental

General Procedures

The mp's were determined with a Buchi apparatus and are uncorrected. A Perkin-Elmer Model 141 MC polarimeter and 10-cm tubes were used for measurement of specific rotations. ^1H NMR spectra were recorded in chloroform-*d* solution at 400 MHz. The ^{13}C NMR spectra were measured in chloroform-*d* solution at 50.31 MHz with a Bruker WP-200 spectrometer. Chemical shifts are given in ppm, and TMS was the internal standard. ^{13}C chemical shifts for sugar aromatic carbons and aglycone carbons for compounds **13** and **14** are not given. The latter were almost identical with those of **12** which are indicated. MS were measured with a Kratos MS80RF instrument fast atom bombardment (FAB). Microanalyzes were performed by the Service Central de Microanalyze du C.N.R.S. Silica gel 60 F_{254} (Merck) activated at 120°C was the support for TLC and for column chromatography.

Methyl 4,6-*O*-Benzylidene-2,3-dideoxy-2-*C*-methyl-3-trifluoroacetamido-α-D-*allo*-hexopyranoside (7)

To a solution of **4** (1 g, 3.6 mmol) in dry pyridine (25 ml) was added hydroxylamine hydrochloride (860 mg, 13.7 mmol) and the mixture was stirred at room temperature for 5 hours. After standard workup a crystalline mixture of *cis* and *trans* oximes **5** (1 g, 95%) was obtained. A 70% solution of Red-Al in dry toluene (4.4 ml, 15.4 mmol) was added to a solution of **5** (1 g, 3.3 mmol) in toluene (10 ml) at −40°C.[5] Stirring was maintained at −40°C for 0.5 hour and then at room temperature for 2 hours. After extractive isolation, the unstable amine **6** (825 mg, 90%) was obtained. Dry pyridine (10 ml) and trifluoroacetic anhydride (1.4 ml, 10 mmol) were successively added to a stirred solution of amine **6** (825 mg, 2.97 mmol) in ether (20 ml) at −40°C. After 5 hours at 0°C, after dissolution in ether, the organic layer was washed, dried over $MgSO_4$ and concentrated *in vacuo*. Flash chromatography, using hexane - ethyl acetate (4:6), gave pure crystalline **7** (1 g, 92%): MP 100~102°C; $[\alpha]_D^{22}$ +11° (*c* 0.82, $CHCl_3$); ^1H NMR δ 7.46~7.50 (5H, m, Ph), 5.58 (1H, s, 7-H), 4.58 (1H, d, $J_{1,2}$=4 Hz, 1-H), 4.53 (1H, m, 3-H), 4.30 (1H, q, $J_{5,6eq}$=5 Hz, J_{gem}=10 Hz, 6-H_{eq}), 3.80 (1H, t, $J_{5,6ax}=J_{gem}$=10 Hz, 6-H_{ax}), 3.78 (1H, m, 5-H), 3.70 (1H, q, $J_{3,4}$=4 Hz, $J_{4,5}$=10 Hz, 4-H), 3.45 (3H, s, OCH_3), 2.20 (1H, m, 2-H).

Anal Calcd for $C_{17}H_{20}F_3NO_5$: C 54.39, H 5.37, N 3.73.
Found: C 54.65, H 5.25, N 3.69.

Methyl 4-*O*-Benzoyl-2-*C*-methyl-2,3,6-trideoxy-3-trifluoroacetamido-β-L-*talo*-hexopyranoside (10)

A suspension of **7** (1.3 g, 3.4 mmol) and *N*-bromosuccinimide (720 mg, 4.1 mmol) in anhydrous

carbon tetrachloride (32 ml) was refluxed overnight in an argon atmosphere. After cooling and filtration through kieselguhr, the organic layer was washed with a solution of sodium thiosulfate. Extractive isolation gave crude **8** which, without further purification, was treated as follows. A mixture of **8** (1 g, 2.2 mmol) and silver fluoride (1.95 g, 15.4 mmol) in dry pyridine (100 ml) was stirred in the dark for 3 days. The resulting mixture was then diluted with ether, filtered through kieselguhr, concentrated and purified by flash chromatography using hexane - ethyl acetate (8:2) giving pure **9** (560 mg, 68%). A mixture of **9** (1 g, 2.7 mmol) in methanol (50 ml) and 5% Pd - C (75 mg) was hydrogenated overnight at atmospheric pressure. Filtration on kieselguhr and concentration gave a residue which was purified by flash chromatography using pentane - ether (9:1). Pure **10** was obtained as a syrup: $[\alpha]_D^{22}$ −25° (*c* 2, $CHCl_3$); ^1H NMR δ 8.17, 7.70 and 7.57 (5H, m, OCOPh), 5.30 (1H, t, $J_{3,4}=J_{4,5}=4$ Hz, 4-H), 4.62 (1H, d, $J_{1,2}=2$ Hz, 1-H), 4.50 (1H, m, 3-H), 4.02 (1H, br q, $J_{5,6eq}=7$ Hz, 5-H), 3.62 (3H, s, OCH_3), 2.40 (1H, m, 2-H), 1.31 (3H, d, $J_{5,6}=7$ Hz, 6-H), 1.10 (3H, d, $J_{2,CH_3}=7$ Hz, 2-CH_3).

Anal Calcd for $C_{17}H_{20}F_3NO_5$: C 54.39, H 5.37, N 3.73.
Found: C 54.27, H 5.55, N 3.59.

7-*O*-(4′-*O*-Benzoyl-2′-*C*-methyl-2′,3′,6′-trideoxy-3′-trifluoroacetamido-α-L-*talo*-pyranosyl)daunomycinone (**12**), 7-*O*-(4′-*O*-Benzoyl-2′-*C*-methyl-2′,3′,6′-trideoxy-3′-trifluoroacetamido-α-L-*galacto*-pyranosyl)daunomycinone (**13**) and 7-*O*-(4′-*O*-Benzoyl-2′-*C*-methyl-2′,3′,6′-trideoxy-3′-trifluoroacetamido-α-L-*talo*-furanosyl)daunomycinone (**14**)

A solution of **10** (500 mg, 1.35 mmol) in 20% acetic acid in water (18 ml) was stirred under reflux for 36 hours. After cooling, the mixture was evaporated below 30°C to give a yellow solid. The latter was dissolved in pyridine (20 ml) and acetic anhydride was added to the solution while keeping the temperature at 0°C. After 5 hours the mixture was worked up to furnish **11** (490 mg, 90%). To a solution of daunomycinone (**3**) (450 mg, 1.15 mmol) and anhydrous pTSA (680 mg, 3.45 mmol) in dry dichloromethane (50 ml) and toluene (50 ml) was added dropwise in an argon atmosphere the acetylated sugar **11** (1 g, 2.30 mmol) in dichloromethane (5 ml). After stirring for 5 hours at room temperature in the dark, the mixture was diluted with dichloromethane (150 ml), washed successively with a 5% aqueous solution of sodium hydrogen carbonate and the organic layer was dried over $MgSO_4$. The residue was flash chromatographed to afford **12** (175 mg, 50%), **13** (105 mg, 30%) and **14** (70 mg, 20%).

Pure **12**: MP 104°C; $[\alpha]_D^{22}$ +64° (*c* 0.44, $CHCl_3$); FAB-MS *m/z* 764 (MNa^+); ^1H NMR δ 8.09, 7.65 and 7.51 (5H, m, OCOPh), 8.03 (1H, d, $J_{1,2}=8$ Hz, 1-H), 7.79 (1H, t, $J_{1,2}=J_{2,3}=8$ Hz, 2-H), 7.40 (1H, d, $J_{2,3}=8$ Hz, 3-H), 6.78 (1H, d, $J_{3,NH}=8$ Hz, NH), 5.40 (1H, br s, 1′-H), 5.30 (1H, br s, 4′-H), 5.20 (1H, br s, 7-H), 4.54 (1H, m, 5′-H), 4.48 (1H, m, 3′-H), 4.12 (1H, s, 9-OH), 4.10 (3H, s, OCH_3), 3.27 (1H, d, $J_{gem}=18$ Hz, 10-H_α), 2.92 (1H, d, $J_{gem}=18$ Hz, 10-H_β), 2.43 (3H, s, 14-H), 2.37 (1H, q, $J_{7,8\alpha}=2$ Hz, $J_{gem}=16$ Hz, 8-H_α), 2.27 (1H, m, 2′-H), 2.20 (1H, d, $J_{gem}=16$ Hz, 8-H_β), 1.30 (3H, d, $J_{5',6'}=7$ Hz, 6′-H), 1.22 (3H, d, 2′-CH_3); ^{13}C NMR δ 211.1 (C-13), 187.3 (C-5, C-12), 161.7 (C-4), 156.2 (C-6), 152.3 (C-11), 134.7 (C-2), 128.6 (C-5a), 120.2 (C-4a, C-3), 119.2 (C-1), 105.7 (C-1′), 77.0 (C-9), 71.9 (C-4′), 70.7 (C-7′), 66.6 (C-5′), 57.0 (OCH_3), 48.9 (C-3′), 35.8 (C-2′), 35.4 (C-8), 33.9 (C-10), 24.4 (C-14), 16.5 (C-6′), 15.3 (2′-CH_3).

Pure **13** showed mp 142°C; $[\alpha]_D^{22}$ +31° (*c* 0.7, $CHCl_3$); FAB-MS *m/z* 764 (MNa^+); ^1H NMR δ 8.13, 7.62 and 7.48 (5H, m, OCOPh), 7.87 (1H, d, $J_{1,2}=8$ Hz, 1-H), 7.73 (1H, t, $J_{1,2}=J_{2,3}=8$ Hz, 2-H), 7.33 (1H, d, $J_{2,3}=8$ Hz, 3-H), 6.54 (1H, d, $J_{3',NH}=8$ Hz, NH), 5.48 (1H, br s, 1′-H), 5.44 (1H, br s, 7-H), 5.23 (1H, br s, 4′-H), 4.52 (1H, m, 3′-H), 4.20 (1H, m, 5′-H), 4.05 (1H, s, 9-OH), 4.02 (3H, s, OCH_3), 3.17 (1H, d, $J_{gem}=18$ Hz, 10-H_α), 2.85 (1H, d, $J_{gem}=18$ Hz, 10-H_β), 2.42 (3H, s, 14-H), 2.33 (1H, q, $J_{7,8\alpha}=2$ Hz, $J_{gem}=16$ Hz, 8-H_α), 2.13 (1H, m, 2′-H), 2.05 (1H, d, $J_{gem}=16$ Hz, 8-H_β), 1.25 (3H, d, $J_{5',6'}=7$ Hz, 6′-H), 0.86 (3H, d, 2′-CH_3); ^{13}C NMR δ 103.5 (C-1′), 71.0 (C-4′), 66.6 (C-5′), 50.6 (C-3′), 34.2 (C-2′), 16.8 (C-6′), 12.3 (2′-CH_3).

Pure **14** was a syrup: $[\alpha]_D^{22}$ +222° (*c* 0.47, $CHCl_3$); FAB-MS *m/z* 764 (MNa^+); ^1H NMR δ 8.06, 7.58 and 7.46 (5H, m, OCOPh), 8.06 (1H, d, $J_{1,2}=8$ Hz, 1-H), 7.82 (1H, t, $J_{1,2}=J_{2,3}=8$ Hz, 2-H), 7.42 (1H, d, $J_{2,3}=8$ Hz, 3-H), 5.68 (1H, br s, 7-H), 5.50 (1H, d, $J_{1,2}=3$ Hz, 1′-H), 5.35 (1H, m, 5′-H), 4.62

(1H, m, 3′-H), 4.45 (1H, m, 4′-H), 4.10 (1H, s, 9-OH), 4.08 (3H, s, OCH_3), 3.27 (1H, d, J_{gem}=18 Hz, 10-H_α), 2.96 (1H, d, J_{gem}=18 Hz, 10-H_β), 2.56 (1H, s, 2′-H), 2.55 (1H, q, $J_{7,8\alpha}$=2 Hz, J_{gem}=16 Hz, 8-H_α), 2.41 (3H, s, 14-H), 1.91 (1H, d, J_{gem}=16 Hz, 8-H_β), 1.27 (3H, d, $J_{5',6'}$=7 Hz, 6′-H), 0.95 (3H, d, 2′-CH_3); ^{13}C NMR δ 100.5 (C-1′), 70.7 (C-5′), 53.3 (C-3′), 42.0 (C-2′), 16.3 (C-6′), 7.3 (2′-CH_3).

7-*O*-(3′-Amino-2′-*C*-methyl-2′,3′,6′-trideoxy-α-L-*talo*-pyranosyl)daunomycinone Hydrochloride (**17**) and 7-*O*-(3′-Amino-2′-*C*-methyl-2′,3′,6′-trideoxy-α-L-*galacto*-pyranosyl)daunomycinone Hydrochloride (**18**)

A solution of **12** (or **13**) (80 mg, 0.18 mmol) in 0.1 N sodium hydroxide in water (6.5 ml) was stirred at room temperature in an argon atmosphere for 10 minutes. The reaction was then quenched by dropwise addition of 0.1 N hydrochloric acid until pH 5. Dilution with dichloromethane was followed by extractive isolation. The crude product was purified by preparative TLC, using dichloromethane - methanol (97 : 3), to afford pure **15** (40 mg, 61%): $[\alpha]_D^{22}$ +189° (*c* 0.11, $CHCl_3$); FAB-MS *m/z* 637 (M^+) or pure **16** (43 mg, 62%): MP 142°C; $[\alpha]_D^{22}$ +206° (*c* 0.44, $CHCl_3$); FAB-MS *m/z* 637 (M^+). To a solution of **15** (or **16**) (12 mg, 0.02 mmol) in methanol (2 ml) was added a 0.1 N solution of barium hydroxide in water (1.2 ml). The mixture was stirred at room temperature for 1.5 hours. The pH was adjusted to 5 by dropwise addition of a 0.1 N solution of hydrochloric acid in water. Dilution with dichloromethane was followed by extractive isolation. The free amine (10 mg, 95%), was treated in dichloromethane solution with a 0.25 N solution of hydrogen chloride in CH_2Cl_2. After evaporation of the solvent, the antibiotic hydrochloride **17** (or **18**) crystallized from methanol - ether.

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