7-O-ALKYL DERIVATIVES OF DAUNOMYCINONE

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Reaction of daunomycinone (*I*) with alcohols and *p*-toluenesulfonic acid produces a mixture (~3 : 1) of its (7*S*)- and (7*R*)-O-alkyl derivatives *II*–*IX*. According to the ^{1}H NMR evidence, the alicyclic ring exists in the $^9H_8$ conformation in (7*R*)-O-alkyl derivatives, on the contrary to (7*S*)-epimers and 7-*epi*-daunomycinone that adopt the $^8H_9$ conformation.

Daunomycinone (*I*) is the aglycone of daunomycin, a glycoside used in the treatment of some forms of cancer[1]. Similarly to the majority of anthracyclinones, *I* itself is biologically inactive[2]. However, active compounds were obtained by 7-O-alkylation of nogalarol, an aglycone of another anthracycline antibiotic nogalamycin, and its 10-demethoxycarbonyl analogs[3]. Remarkable antileukemic effects exhibits the semisynthetic (10*S*)-methoxydaunomycin[4]. Some activity was also found in (7*S*)-O-hydroxyalkyl derivatives of daunomycinone during the preliminary tests[5]. That prompted us to adopt the same procedure[5] to the preparation of 7-O-alkyl derivatives of *I*. These compounds are also suitable models for the study of conformation and spectroscopic properties of anthracycline compounds. The simplicity of their spectra in comparison with that of glycosides is an advantage in such a study.

7-O-Methyl-4-desmethyldaunomycinone was prepared[6] by demethylation of daunomycinone trimethyl ether[7]; other 7-O-methylated compounds were prepared as intermediates during the total synthesis of anthracyclinones[8,9]. Selective methylation of phenolic groups was also described in some related compounds[10–12].

Two main products are always formed in the reaction of daunomycinone with the corresponding alcohol and *p*-toluenesulfonic acid in boiling benzene–xylene mixture (Table I). Their physical properties (Table II) are sufficiently different. Their mass spectra (Table III) contain besides the molecular ion an ion M – ROH, *m*/*z* 380, (where R corresponds to the alkyl of the alcohol used for the alkylation) and the same fragment series that is also present in the spectrum of daunomycinone. The spectra of corresponding pairs (*II*–*III*, *IV*–*V*, *VI*–*VII*, *VIII*–*IX*) differ in the relative peak intensities only. That shows that these compounds are isomeric monoalkyl derivatives of daunomycinone. This conclusion is supported by proton chemical

TABLE I

Reaction of daunomycinone with alcohols

Alcohol	Yield %	Temperature °C	Time h	Products	Ratio[a] 7*S*/7*R*
CH_3OH	45	120	48	*II*, *III*	3·5
C_2H_5OH	26	130	96	*IV*, *V*	2·5
$(CH_3)_2CHOH$	37	120	36	*VI*, *VII*	2·7
$(CH_3)_2CHCH_2OH$	48	120	4	*VIII*, *IX*	3·0

[a] Calculation based on the isolated compounds.

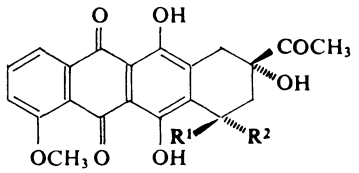
TABLE II

Physical properties of compounds *II*—*IX*

Compound	M.p., °C	$[\alpha]_D^{20}$ (conc.)[a]	R_F[b]	Formula (m.w.)	Calculated/Found	
					% C	% H
II	181—183	+134 (0·042)	0·59	$C_{22}H_{20}O_8$ (412·4)	64·07 64·25	4·89 4·62
III	205—209	0 (0·021)	0·48	$C_{22}H_{20}O_8$ (412·4)	64·07 64·20	4·89 4·77
IV	195—197	+175 (0·016)	0·62	$C_{23}H_{22}O_8$ (426·4)	64·78 64·96	5·20 5·37
V	206—209	− 60 (0·027)	0·46	$C_{23}H_{22}O_8$ (426·4)	64·78 64·91	5·20 5·30
VI	133—135	+280 (0·02)	0·62	$C_{24}H_{24}O_8$ (440·4)	65·45 64·60	5·49 5·65
VII	204—207	+ 80 (0·02)	0·48	$C_{24}H_{24}O_8$ (440·4)	65·45 64·58	5·49 5·36
VIII	158—160	+162 (0·016)	0·61	$C_{25}H_{26}O_8$ (454·5)	66·07 66·28	5·77 5·85
IX	204—206	+189 (0·019)	0·52	$C_{25}H_{26}O_8$ (454·5)	66·07 66·25	5·77 5·70

[a] In chloroform; [b] DC Fertigplatten Kieselgel 60 Merck, system benzene–chloroform–ethyl acetate–methanol 7 : 7 : 3 : 1.

shifts (Table IV). From them it is evident that the isomers of different polarity can be divided in two groups – *II*, *IV*, *VI*, *VIII* and *III*, *V*, *VII*, *IX*. The differences between



I, $R^1 = H$; $R^2 = OH$
II, $R^1 = H$; $R^2 = OCH_3$
III, $R^1 = OCH_3$; $R^2 = H$
IV, $R^1 = H$, $R^2 = OC_2H_5$
V, $R^1 = OC_2H_5$; $R^2 = H$
VI, $R^1 = H$; $R^2 = OCH(CH_3)_2$
VII, $R^1 = OCH(CH_3)_2$; $R^2 = H$
VIII, $R^1 = H$; $R^2 = OCH_2CH(CH_3)_2$
IX, $R^1 = OCH_2CH(CH_3)_2$, $R^2 = H$
X, $R^1 = OH$; $R^2 = H$

the corresponding protons are similar in all pairs. Variability of the chemical shift of the $H_{(7)}$ proton can be ascribed to different alkyls. The different width of the $H_{(7)}$ multiplet in both groups (5·5–6·1 Hz and 8·6–11·0 Hz, respectively) becomes evident from the inspection of proton-proton coupling constants (Table V). A long-range

TABLE III
Mass spectra of compounds *II*–*IX*

m/z	Composition[a]	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*
454[b]	$C_{25}H_{26}O_8$	–	–	–	–	–	–	16	5
440[b]	$C_{24}H_{24}O_8$	–	–	–	–	13	4	–	–
426[b]	$C_{23}H_{22}O_8$	–	–	28	6	–	–	–	–
412[b]	$C_{22}H_{20}O_8$	34	8	–	–	–	–	–	–
382	$C_{21}H_{18}O_7$	5[c]	4[c]	13	8	22	18	30	17
380	$C_{21}H_{16}O_7$	5	7	3[c]	4[c]	4[c]	4[c]	7[c]	6[c]
362	$C_{21}H_{14}O_6$	83	30	36	28	46	38	69	64
344	$C_{21}H_{12}O_5$	30	14	10	11	12	19	36	41
339	$C_{19}H_{15}O_6$	19[c]	21[c]	24[c]	30[c]	46	53	60	60
337	$C_{19}H_{13}O_6$	100	100	100	100	100	100	100	100
321	$C_{19}H_{13}O_5$	16[c]	17[c]	24	30	35	53	64	76
319	$C_{19}H_{11}O_5$	23	17	21	23	29	35	40	35
309	$C_{18}H_{13}O_5$	72	42	63	43	63	44	70	41
301	$C_{19}H_9O_4$	19	10	6[c]	9[c]	7[c]	15[c]	29	31
217	$C_{12}H_9O_4$	42	28	35	29	32	34	43	31
43	C_2H_3O	75	38	56	57	60	94	67	72

[a] High resolution measurement; [b] molecular ion; [c] high resolution measurement not performed.

coupling is observed between the equatorial protons $H_{(8e)}$ and $H_{(10e)}$ existing in favorable W-arrangement[7,13,14]. This coupling was indeed observed in both series; in the former one, however, these equatorial protons resonate in the lower fields than their axial counterparts, on the contrary to the other series. The course of the circular dichroism curve in the region 280–350 nm is with compounds *II* and *VI* similar to daunomycinone[15] (*II*: $\Delta\varepsilon_{284}$ −0·61, $\Delta\varepsilon_{308}$ + 0·60, $\Delta\varepsilon_{348}$ +0·31; *VI*: $\Delta\varepsilon_{284}$ −1·04;

TABLE IV

Chemical shifts (δ-scale, ppm) of selected protons in ^{1}H NMR spectra of compounds *II*–*IX*

Compound	Alicyclic ring[a]					Side chain[b]			
	7	8a	8e	10a	10e	14	α	β	γ
II	4·95	1·96	2·41	2·93	3·24	2·43	3·62	—	—
IV	5·07	1·95	2·40	2·95	3·24	2·43	3·86	1·25	—
VI	5·15	1·96	2·33	2·93	3·22	2·42	4·17	1·25, 1·29	—
VIII	5·02	1·93	2·38	2·94	3·23	2·43	3·58	1·88	0·90
III	5·01	2·56	2·08	3·29	3·00	2·37	3·41	—	—
V	5·10	2·54	2·07	3·32	2·99	2·39	3·66	1·16	—
VII	5·19	2·47	2·08	3·29	3·00	2·41	3·90	1·11, 1·21	—
IX	5·07	2·54	2·05	3·28	3·00	2·39	3·32	1·77	0·86

[a] a axial, e equatorial; [b] relative position in the side chain.

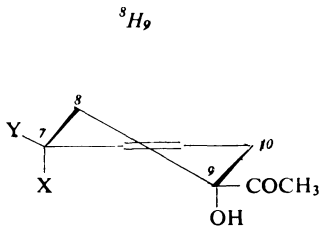
TABLE V

Selected coupling constants (Hz) of alicyclic ring protons in compounds *II*–*IX*

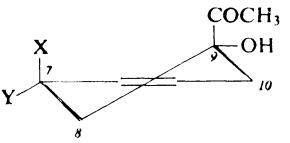
Compound	$\sum J_7$[a]	7,8a	7,8e	8a,8e	8e,10e	10a,10e
II	5·5	3·7	1·8	15·3	1·2	18·9
IV	6·1	3·7	2·4	14·7	1·2	18·9
VI	5·5	3·7	1·8	14·7	1·2	19·0
VIII	5·5	3·7	1·8	14·7	1·2	19·5
III	11·0	4·9	6·1	14·6	1·0[b]	17·9
V	11·0	4·9	6·1	14·6	1·0[b]	18·3
VII	9·8	3·7	6·1	14·6	1·0[b]	17·1
IX	8·6	3·7	4·9	14·6	1·0[b]	18·9

[a] $J_{7,8a} + J_{7,8e}$, a axial, e equatorial H; [b] estimated from the linewidth and confirmed by decoupling experiment.

$\Delta\varepsilon_{305}$ +0·55, $\Delta\varepsilon_{350}$ +0·55). Therefore, they can be formulated as (7*S*)-derivatives. CD spectra of compounds *III* and *VII* approach a mirror image of the spectra of compounds *II* and *VI* (*III*: $\Delta\varepsilon_{290}$ +0·72, $\Delta\varepsilon_{350}$ −0·13; *VII*: $\Delta\varepsilon_{290}$ +0·44, $\Delta\varepsilon_{350}$ −0·07) what indicates that they are their 7-epimers. For the linewidth of the $H_{(7)}$ proton in 7-*epi*-daunomycinone (ref.[16-18]) (X) are reported values 17−18 Hz, for the 7-epimers of other anthracyclinones[9,19] they amount 15−16 Hz. However, the observed values $J_{7,8e}$ and $J_{7,8a}$ (Table V) are too small for an axial-axial coupling in the conformation $^8H_9$ (Scheme 1). Therefore, the proton $H_{(7)}$ must be also pseudo-



$^8H_9$



$^9H_8$

I, *II*, *IV*, *VI*, *VIII*: X = OH or OR, Y = H
X: X = H, Y = OH

III, *V*, *VII*, *IX*: X = OR, Y = H

SCHEME 1

equatorial in the discussed compounds. These facts can be accomodated in the second of the two possible conformations of the alicyclic ring, $^9H_8$ (Scheme 1). On that basis it is possible to interpret so far observed facts. The axial methyl keto group is in this conformation more exposed to the ring effect of the aromatic ring and therefore resonates in slightly higher field (Table IV, proton 14). The change in the chemical shifts of protons at $C_{(8)}$ and $C_{(10)}$ can be explained by their different orientation with respect to the hydroxyl at $C_{(9)}$; the equatorially oriented OH group in conformation $^9H_8$ exerts large effect on $H_{(8e)}$ and $H_{(10e)}$. It remains to explain the different values of $J_{7,8a}$ and $J_{7,8e}$ in both conformations since the relevant dihedral angles are the same. It is known that the magnitude of the vicinal coupling constants in the fragment —OCHCH$_2$— in saturated systems is influenced not only by the electronegativity of substituents but also by their orientation[20]. An axially oriented group decreases the magnitude of this coupling. Our examples represent an evident extension of this rule to the case of a substituent on the atom next to the methylene group. An axial hydroxyl at $C_{(9)}$ in (7*S*)-derivatives in the conformation $^8H_9$ decreases the magnitude of the coupling whereas with equatorial $C_{(9)}$—OH in conformation $^9H_8$ their values are normal. The mentioned conformation was already found in several derivatives of anthracyclinones[3,5]. We have found in our previous work[5] that an increase in size of the side chain substituent at $C_{(9)}$ causes the change

of conformation from $^{9}H_{8}$ to $^{8}H_{9}$ in the (7*R*)-epimers. Indeed, the multiplet of the $H_{(7)}$ in the ^{1}H NMR spectrum of compound *XI* prepared by acetalization of *III* by ethylene glycol, has the width of 14·7 Hz corresponding to an axial proton in the conformation $^{8}H_{9}$. Thus, during the described alkylation procedure both inversion and retention of configuration at $C_{(7)}$ take place. It can be expected on the analogy with the similar cases in other anthracyclinones[3,21] that the intermediates of this reaction are also 7-O-esters.

Compounds *II*, *IV*, *V*, *VII*–*IX* inhibit the growth of *Bacillus subtilis* (diameter of inhibition zones in mm caused by 0·1 mg of compounds: 20, 17, 16, 20, 18, 17; daunomycin: 29). On the contrary to 7-O-hydroxyalkyl derivatives of daunomycinone, where biologically active were the (7*S*)-derivatives only[5], we also found activity in some members of the (7*R*)-series.

EXPERIMENTAL

Melting points were determined in a Kofler apparatus. Optical rotations were measured using an automatic polarimeter Bendix Ericson. CD spectra (in dioxan) were measured on a Roussel Jouan CD 185 Dichrograph instrument. Mass spectra were studied on a Varian MAT 311 mass spectrometer (energy of ionizing electrons 70 eV, ionizing current 1 mA, ion source temperature 200°C, direct inlet at 150–170°C). ^{1}H NMR spectra were measured on a Jeol FX-60 spectrometer (FT mode, 59·797 MHz) in deuteriochloroform at 25°C. Tetramethylsilane was used as an internal standard. Chemical shifts were calculated from the digitally obtained address differences (±0·02 ppm). They are given in the δ-scale. The reported R_F values arise from thin layer chromatography on DC-Fertigplatten (Kieselgel 60, Merck) in the system benzene–chloroform–ethyl acetate–methanol 7 : 7 : 3 : 1. Biological activity was assayed by agar diffusion test against *Bacillus subtilis*.

(7*S*)-9-Acetyl-4,7-dimethoxy-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenequinone (*II*)

Daunomycinone (*I*, 100 mg) was dissolved in the mixture of benzene (6 ml) and xylene (8 ml); methanol (25 ml) and *p*-toluenesulfonic acid (50 mg) were added. The reaction took place for 48 h at 120°C, then water was added and the mixture was extracted by chloroform. Solvent was removed and the residue was subjected to column chromatography on silica gel (according to Pitra); the eluent was benzene. The mixture of *II* and *III* was separated by preparative thin layer chromatography on Silufol 20 in the above mentioned system. Similarly were prepared compounds *IV*–*IX* (Tables I–V).

Acetal of (7*R*)-9-acetyl-4,7-dimethoxy-7,8,9,10-tetrahydro-6,9,11-trihydroxy-5,12-naphthacenequinone (*XI*)

Compound *III* (5 mg) was dissolved in benzene (4 ml) and 1,2-ethandiol (0·5 ml) and *p*-toluenesulfonic acid (0·2 mg) were added. The mixture was heated 8 min at 130°C and then poured into water and extracted by chloroform. Solvents were removed and the residue was separated by thin layer chromatography on Alufolien Kieselgel (Merck) in the system benzene–chloroform–methanol 25 : 65 : 8. The yield of *XI* was 4·5 mg, m.p. 193–196°C; R_F 0·58; $[\alpha]_D^{20}$ −153° (*c* 0·12, chloroform). Mass spectrum *m/z* (% of relative intensity, composition): 456 (0·4, $C_{24}H_{24}O_9$,

M^+), 406 (7, $C_{23}H_{18}O_7$), 391 (11, $C_{22}H_{15}O_7$), 362 (0·1), 347 (0·2), 319 (0·2), 217 (0·2), 87 (100, $C_4H_7O_2$). ^{1}H NMR spectrum: 1·47 s (3 H), 1·98 dd (J = 7·4 and 14·7 Hz, 1 H), 2·47 dd (J = = 7·3 and 14·7 Hz, 1 H), 2·86 d (J = 18·3 Hz, 1 H), 3·22 d (J = 18·3 Hz, 1 H), 3·47 s (3 H), 4·10 s (7 H), 4·99 mt (1 H), 7·35 dd (J = 7·3 and 1·2 Hz, 1 H), 7·73 t (J = 7·3 Hz, 1 H), 8·02 dd (J = 7·3 and 1·2 Hz, 1 H), 13·37 s (1 H), 13·94 s (1 H).

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