von Desgluco-C_1 (=Acetyldigoxosid) in 2 Formen (α und β) beweisen die Bindung einer Acetylgruppe zu der vierten Digitoxoseeinheit, wofür auch die Ergebnisse der partiellen Hydrolyse[6] von Acetyldigoxosid sprechen. Nach dieser Hydrolyse wurden neben dem Ausgangsglykosid (0,30; 0,90), Digoxigenin (0,28; 0,32), Digoxigenin-mono (0,22)- und bisdigitoxoside (0,20; 0,39), sowie das Digoxin (0,18; 0,45) nachgewiesen (System c und d). Durch Behandlung mit schwachem Alkali wurde nur das Ausgangsglykosid gespalten und es entstand Digoxosid. Aus der molekularen Drehungsdifferenz von genuinen Glykosid und Acetyldigoxosid ist zu schliessen, dass die Glucose β-glykosidisch an das vierte Digitoxosemolekül gebunden ist. Aufgrund dieser Befunde muss dem neuen genuinen Glykosid C_1 die Struktur **I** zukommen. Glucoacetyldigoxosid ist in *Digitalis-schischkinii*-Blättern 0,04–0,05% enthalten. Sein Gehalt übertrifft meist den an Lanatosid E und D und stellt somit das vierte primäre Hauptglykosid der Droge dar.

Glykosid C_2, das sich in den am stärksten polaren Glykoside enthaltenden Fraktionen der Silikagelsäule befindet, konnte durch weitere intensive Chromatographie an Cellulose mit System b und an Silikagel mit Äthylacetat + 2–20% Äthanol und mit Chloroform nicht vollkommen von Desacetyllanatosid C getrennt werden, und die isolierte Substanz war noch stark verunreinigt. Das Glykosid liegt in den Chromatogrammen dicht über (System a) bzw. unter (System b) Desacetyllanatosid C und zeigt den gleichen R_f-Wert mit desacetyliertem C_1 (=Glucodigoxosid). Identifizierung der Substanz als Glucodigoxosid **II** erfolgte über nach Fermentation erhaltenes sekundäres Glykosid. Aus der fermentierten Mischung über präparative DC auf Kieselgur G mit System d abgetrenntes Glykosid gibt mit dem des Digoxosids deckungsgleiches IR-Spektrum. Glucodigoxosid kommt in der Droge in Mengen von ca. 0,003% vor.

Mit der Isolierung dieser Glucosido-tetradigitoxoside ist gezeigt worden, dass in der Pflanze nicht nur Mono-Bis- und Tridigitoxoside, sondern auch Tetradigitoxoside der Digitalisgenine mit endständiger Glucose verknüpft vorkommen.

Glucoacetyldigoxosid (Glykosid C_1): $C_{55}H_{86}O_{23}.3H_2O$ (1169.34). Ber.: C 56.49 H 7.93 Glucose 15.41; Gef.: C 56.30 H 7.20 Glucose 16.18, Schmp. 230–242 °C, $[\alpha]_D^{26} = +16.9°$ (c=0.213 in Pyridin) $[M]_D = +197.6°$, UV (Methanol) λ_{max} 218–219 nm ($\log\varepsilon$ 4.15). α/β-Acetyldigoxosid: $C_{49}H_{76}O_{18}.1H_2O$ (971.16) Ber.: C 60.60 H 810; Gef.: C 60.92 H 7.29, Schmp. 173–175 °C/268–273 °C, $[\alpha]_D^{25} = +27.1°$ (c=0.267 in Pyridin), $[M]_D = +263.2°$.

Digoxosid: $C_{47}H_{74}O_{17}.1H_2O$ (929.13) Ber.: C 60.76 H 8.25; Gef.: C 60.68 H 7.85, Schmp. 265–271 °C, $[\alpha]_D^{25} = +18.1°$ (c=0.418 in Pyridin).

1 Wir danken Herrn Dr. F. Kaiser, Mannheim, für die Überlassung einer Probe von Digoxosid und Herrn Prof. Dr. H. Wagner, München, für die Durchführung der Elementaranalysen und optischer Drehungen.

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Synthesis and antitumour activity of new daunorubicin and adriamycin analogues

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Summary. A new synthetic procedure for the preparation of daunorubicin and adriamycin analogues bearing different substituents on ring D, has been developed. The new compounds display outstanding efficacy against experimental tumours of mice.

Clinical usefulness of adriamycin (**I**) has prompted the study of new analogues of the antitumour anthracyclines with hopefully broader spectrum of activity and/or reduced cardiotoxicity[1,2]. New derivates modified in the C-9 side chain and in the sugar moiety have already excited pharmacological interest[3–5]. However, it has recently been found that 4-demethoxydaunorubicin **VIa** and 4-demethoxyadriamycin **VIIa,** obtained by total synthesis of the aglycone moiety, according to Wong's synthetic sequence[6], display a considerable antitumour activity on experimental tumours of laboratory animals[7,8].

Synthetic approaches to the daunomycinone system have been developed in different laboratories after the original synthesis of racemic 4-demethoxy-7-O-methyldaunomycinone carried out by the Canadian authors[6]. These approaches include both Diels-Alder type reactions for the building of the tetracyclic chromophore[9,10] as well as studies based on the Friedel and Crafts acylation of appropriate tetralines with phthalic acid derivatives[11–13]. Racemic compounds were invariably obtained. Further developments have also been recorded in the glycosydation of the anthracyclinones[2].

A simplified synthetic procedure for optically active **Va** has been developed and is reported here. This procedure is based on the direct formation of 7-deoxy-4-demethoxydaunomycinone **IIIa** by condensation of the optically active tetralin **II** with phthalic anhydride, a reaction which is accompanied by an outstanding preservation of the chiral centre, notwithstanding the harsh conditions employed. In the present communication, we also report on the antitumour activity of a group of new daunorubicin and adriamycin analogues (**VI-f** and **VIIb**) carrying different substituents on the aromatic ring and synthesized according to this general procedure.

Chemistry. (S)-(-)-6-acetyl-1,4-dimethoxy-6-hydroxytetralin[14] (**II,** 4 g, 16 mmoles), phthalic anhydride (4 g, 27 mmoles), NaCl (8 g) and $AlCl_3$ (40 g) were thoroughly mixed and transferred into a flask immersed in an oil bath at 180 °C. The resulting melt was stirred for 2 min and after cooling the solid was treated with an excess of a saturated aqueous solution of oxalic acid. The suspension was extracted with chloroform and the extract was evaporated to give a residue which was taken up in ether to give 4 g (11.3 mmoles) of **IIIa,** m.p. 228–230, $[\alpha]_D^{20} - 87°$ (c=0.1 $CHCl_3$) λmax 460, 486, 520 nm ($CHCl_3$). This product was ketalized at C-13 with ethylene glycol (6 ml) in benzene (200 ml), in the presence of p-toluenesulphonic acid (0.2 g), for 5 h at reflux. The crude ketal was dissolved in a mixture

of chloroform (250 ml), carbon tetrachloride (100 ml) and water (200 ml), and treated at 80 °C for 1 h with bromine (4 g) and 2-2′-azo-bis-isobutyrronitrile (1 g). The organic layer was washed repeatedly, evaporated in vacuo, and the residue, mostly 4-demethoxy-7-epidaunomycinone, was treated overnight with trifluoroacetic acid and then with an excess of a 4% concentrated ammonium hydroxide in acetone to give, after chromatography on silicagel, 4-demethoxydaunomycinone[7] (**IVa**), m.p. 184–186 °, $[\alpha]_D^{20}$ +170° (c=0.1 dioxane), λmax 458, 484, 518 nm ($CHCl_3$) (1.2 g, 3.25 mmoles).

The aglycone (1 g) and 2,3,6-trideoxy-3-trifluoroacetamido-4-O-trifluoro acetyl-α-L-lyxo-hexo-pyranosyl chloride[15] (1.2 g) were dissolved in dichloromethane (150 ml), then silver triflate (1 g) in diethyl ether (20 ml) was added, in the dark and in 10 min, at room temperature. After 20 min, excess $NaHCO_3$ solution was added, the organic layer was evaporated, the residue was taken up in methanol (50 ml) and refluxed 30 min. Evaporation of the solvent left a residue that was chromatographed on a short silicagel column (chloroform/acetone – 95/5 as eluent) to give α-N-trifluoroacetyl-4-demethoxydaunorubicin (0.8 g)[7] that was dissolved in 0.1 N NaOH (55 ml). After 30 min the solution was brought to pH 8 with HCl and extracted with chloroform. The solution was concentrated to 10 ml and methanolic HCl was added to pH 4.5. Addition of ether gave a red precipitate of 4-demethoxydaunorubicin hydrochloride[7] (0.5 g).

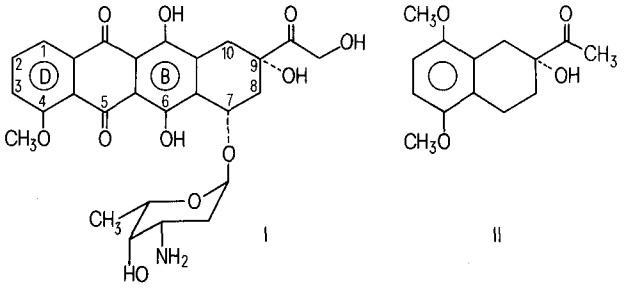
This procedure, which greatly simplifies the synthesis of **IVa,** has been found suitable for the preparation of the analogues **IVb,** m.p. 208–210 °, λmax 460, 484, 518 nm; **IVc,** m.p. 143–145 °, λmax 464, 492, 526 nm; **IVd,** m.p. 195–197 °, λmax 474, 492, 528 nm; **IVe,** m.p. 160–162 °, λmax 478, 506, 542 nm; **IVf,** m.p. 185–187 °, λmax 467, 477, 511 nm. The condensation of the sterically hindered 3,6-dimethyl- and 3,6-dichlorophthalic anhydrides required somewhat higher temperatures and longer times and accordingly a small amount of racemic material was formed and could eventually be eliminated by crystallization, being less soluble that the optically active form. Since, however, it has already been proved[16] that racemic 4-demethoxydaunomycinone could be clearly resolved through the condensation with 2,3,6-trideoxy-3-trifluoroacetamido-4-0-trifluoroacetyl-α-L-lyxo-hexo-pyranosyl chloride in the presence of silver triflate to the 7(S), 9(S)-α-glycoside and to the 7(R), 9(R)-β-glycoside, easily isolated in pure form by chromatography, it was found convenient to use this procedure with all the daunomycinone analogues synthesized, irrespective of their optical purity. Subsequent chromatographic separation of the N-trifluoroacetyl-α-glycosides (**Va-f**) from the accompanying small amounts of 7(R), 9(R)-β-glycosides, followed by alkaline hydrolysis of the trifluoroacetamido group, yielded the optically pure daunorubicin analogues **VIa-f.** Conversion of **VIb** to **VIIb** was performed via bromination at C-14 and nucleophilic substitution of halogen with hydroxy group[17]. All compounds displayed chromatographic and spectroscopic properties consistent with the attributed structures.

Biological data. All substituted 4-demethoxydaunorubicins **VIb-f** displayed a high level of cytotoxic activity, although inferior to that of **VIa** (table). Similarly, **VIIb** appeared less effective than **VIIa,** but still more active than adriamycin itself. With the exception of the halogen-substituted compounds, all derivatives presented in the table displayed activity on L1210 leukemia in mice. In the daunorubicin series, compounds **VIb** and **VId** appeared noteworthy, in addition to already known **VIa,** as regards to the antitumour efficacy in vivo. In the adriamycin series, outstanding antitumour properties were exhibited by **VIIb,** whose effect on the survival time of tumour-bearing animals was clearly superior to that of adriamycin. This compound was also more effective than the corresponding daunorubicin analogue **VIb.** Derivatives **VIb, VId** and **VIe** were also tested on P388 leukemia in mice in comparison with daunorubicin and **VIa.** Compound **VIb** was about twice as potent as daunorubicin, the 1,4-dimethyl analogue **VId** appearing

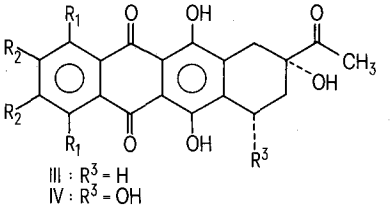
Cytotoxic and antitumour activity of the new analogues compared with daunorubicin and adriamycin

Compound	Cytotoxicity[a] (EC_{50}, ng/ml)	L 1210 leukemia in mice[b]		P 388 leukemia in mice[c]	
		Dose[e]	AST[d]	Dose[e]	AST[d]
Daunorubicin	10.00	2.9	144	4.0	205
VIa	0.15	1.0	150	0.7	200
VIb	5.80	1.25	131	2.0	230
VIc	25.00	33.7	111		
VId	10.05	6.6	147	10.0	165
VIe	7.15	20.0	116	22.5	140
VIf	27.00	10.0	135		
Adriamycin	15.00	2.9	141		
VIIa	0.10	0.5	166		
VIIb	7.00	4.4	173		

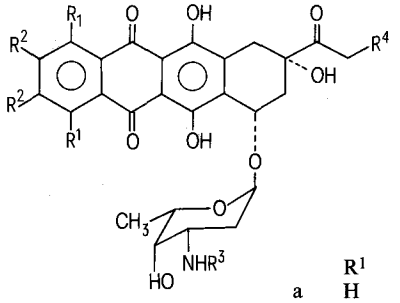
[a] Inhibition of colony-forming ability of cultured HeLa cells after 24 h exposure to the drug. [b] Tumour inoculum 10^5 cells, i.p. [c] Optimal doses, showing no toxicity (treatment i.p. on day 1, mg/kg of b. wt). [d] Average survival time expressed as percent of untreated controls. [e] Tumour inoculum 10^6 cells, i.p.



I II



III : R^3 = H
IV : R^3 = OH



V: R^3 = $COCF_3$, R^4 = H
VI: R^3 = R^4 = H
VII: R^3 = H, R^4 = OH

	R^1	R^2
a	H	H
b	H	CH_3
c	H	Cl
d	CH_3	H
e	Cl	H
f	H	–CH=CH–CH=CH–

instead less potent and less active than daunorubicin, **VIa** and **VIb.**

The biological properties of the new analogues indicate that considerable modification on ring D of the antitumour anthracyclines is compatible with the exhibition of antitumour efficacy. In particular, the 2,3-dimethyl-4-demethoxy analogue showed promising results in the animal tests.

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Naphthoquinone derivatives from the fungus *Hendersonula toruloidea*

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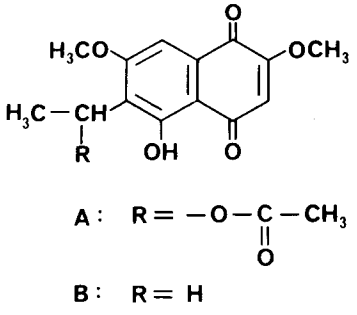
Summary. 2 yellow naphthoquinone derivatives were isolated from several pathogenic strains of *Hendersonula toruloidea* Nattrass. They were identified as 2,7-dimethoxy-6-ethyl-5-hydroxy-1,4-naphthoquinone and 2,7-dimethoxy-5-hydroxy-6-(1-acetoxyethyl)-1,4-naphthoquinone by means of physico-chemical methods.

A fungus isolated from a patient (foot) was found to produce yellow crystalline material on malt agar. The fungus (CBS 131.78) was provided by M.K. Moore of St. John's Hospital for Diseases of the Skin in London and identified as *Hendersonula toruloidea* Nattrass by G.A. de Vries of the Department of Medical Mycology of our institute. The strain M 56 (CBS 145.78 = IMI 198935), one of several other isolates from patients supplied by C.K. Campbell of the M.R.C. Unit on the Experimental Pathology of the Skin in Birmingham, showed the same phenomenon as described above. TLC of an ethyl acetate extract of a culture of both strains revealed the presence of 2 yellow main pigments. Since only 1 pigment formed by *H. toruloidea* has been described[4], it was decided to investigate the nature of the 2 metabolites.

Each strain was grown on malt agar in 100 petri dishes for 20 days at 24 °C. Cultures were extracted with ethyl acetate. Purification was accomplished by means of column chromatography and preparative TLC using toluene/acetone (85:15, v/v) as the developing system. Merck silica gel 60 26×3 cm columns and Merck 2 mm silica gel thick-layer plates were used. Separation of the 2 pigments was successful on the silica gel plates.

Yellow bands comprising compound A, R_f 0.43–0.52 and compound B, R_f 0.53–0.63 were scraped off and eluted with chloroform/methanol (2:1, v/v). A and B were obtained as orange needles by twice recrystallizing from toluene/light petroleum 60–80 °C (1:1, v/v). A and B showed identical UV-visible spectra indicating the same chromophoric system for both pigments. The absorptions measured were similar to those described[5] for the substance originally isolated from *H. toruloidea.* Further study (MS, IR and PMR) revealed that B was identical with the known *Hendersonula* pigment, which was identified as 2,7-dimethoxy-6-ethyl-5-hydroxy-1,4-naphthoquinone (2,7-dimethoxy-6-ethyljuglone, $C_{14}H_{14}O_5$). High resolution MS of A gave the formula $C_{16}H_{16}O_7$. Important peaks were observed at m/e 278 ($M-CH_2CO$) and m/e 260 ($M-CH_3COOH$). The IR-spectrum of A in CCl_4 compared with that of compound B



A: R = —O—C(=O)—CH3

B: R = H

Formation of compounds A and B by other strains of *H. toruloidea*

Strain No.	Origin	Yield mg/20 petri dishes A	B
CBS 204.33	Plant	–	–
CBS 251.49	Plant	–	–
CBS 136.77	Man	1.4	0.4
CBS 137.77	Cow	10.4	1.7
CBS 661.77*	Man	–	–
CBS 662.77*	Man	0.2	–
M 38**	Man	–	–
M 48**	Man	0.2	0.9
M 52**	Man	–	–

* Isolates from M.K. Moore; ** isolates from C.K. Campbell.