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Potential Antitumor Agents, XIV[1)]

1,8-Disubstituted Anthraquinones[+)]

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The synthesis of new derivatives, starting from 1,8-dihydroxy- and 1,8-dichloro-anthraquinone, is reported. The succinic esters **9, 10** and the monofunctional alkylating agent **12** showed significant antitumor activity in mice implanted with Ehrlich ascites tumor cells.

Potentiell tumorhemmende Substanzen, 14. Mitt.: 1,8-Disubstituierte Anthrachinone

Es wird über die Synthese neuer Derivate von 1,8-Dihydroxy- und 1,8-Dichloranthrachinon berichtet. Die Bernsteinsäureester **9** und **10** und das monofunktionelle Alkylans **12** zeigten eine signifikante tumorhemmende Wirkung bei Mäusen mit implantierten Ehrlich ascites Tumorzellen.

The anthracycline antibiotics are of major clinical importance in the treatment of many solid tumors and leukemias. As these drugs cause cumulative, dose-dependent and irreversible cardiomyopathy, many efforts have been made in the search of new, less toxic analogs. Based on the hypothesis that the amino sugar portion is responsible for this cardiotoxicity[2)], several papers have been devoted to the synthesis and antitumor activity of aminoanthraquinone derivatives[3-6)].

With the aim of studying the minimum structural requirements for the antitumor activity of anthraquinone derivatives, we planned the synthesis of new compounds arising from 1,8-dihydroxy- **(1)** and 1,8-dichloro-anthraquinone **(2)**.

Compound **1** is a well known cathartic agent which by reaction with the appropriate acyl chloride gave the esters **3–10**: the structure of these compounds was determined on the basis of the analytical and spectroscopic data (Tables 1, 2). The mass spectrum of

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compounds **3–5** does not show an evident molecular ion peak, while the ^{1}H-NMR spectrum agrees perfectly with the assigned structures (Table 2).

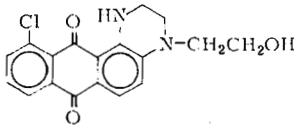
The reaction of compound **2** with diethanolamine in order to prepare the corresponding nitrogen mustard, yielded the mono-alkylated derivative **11**. This result was not quite unexpected since it is well known that dealkylation occurs under oxidative conditions[8,9].

Table 1: *Anthraquinone Derivatives from the anthraquinones* **1, 2**

Compd.	Substituents in: 1	8	Formula (mw)	Calcd. / Found C	H	N	mp °C	Recryst. solvent	$\nu_{max.}$ (cm^{-1})	% T/C at 200 mg/kg
3	$OCOCH_3$	$OCOCH_3$			(7)				1755, 1675, 1665, 1590	
4	$OCOCH_2CH_3$	$OCOCH_2CH_3$	$C_{20}H_{16}O_6$ (352.3)	68.2 / 68.3	4.58 / 4.46	–	163–165	EtOH	1760, 1670, 1590, 1320	
5	$OCO(CH_2)_2CH_3$	$OCO(CH_2)_2CH_3$	$C_{22}H_{20}O_6$ (380.4)	69.5 / 69.8	5.30 / 5.46	–	149–150	EtOH	1765, 1675, 1590, 1320	
6	OCOAd[+]	OCOAd[+]	$C_{36}H_{36}O_6$ (564.6)	76.6 / 76.8	6.43 / 6.56	–	292–295	Acetone	1750, 1670, 1590, 1320	
7	OH	OSO_2CH_3	$C_{15}H_{10}O_6S$ (318.3)	56.6 / 56.5	3.17 / 3.01	–	184–186	EtOH	1665, 1640, 1590, 1280	
8	OSO_2CH_3	OSO_2CH_3	$C_{16}H_{12}O_8S_2$ (396.4)	48.5 / 48.7	3.05 / 2.98	–	234–238	MeOH	1675, 1590, 1170, 955	
9	OH	$OCO(CH_2)_2COOEt$	$C_{20}H_{16}O_7$ (368.3)	65.2 / 65.2	4.38 / 4.29	–	139–140	EtOH	1760, 1725, 1640, 1590	130
10	$OCO(CH_2)_2COOEt$	$OCO(CH_2)_2COOEt$	$C_{26}H_{24}O_{10}$ (496.4)	62.9 / 62.8	4.87 / 4.93	–	93–95	EtOH	1760, 1735, 1670, 1590	150
11	Cl	$NHCH_2CH_2OH$	$C_{16}H_{12}ClNO_3$ (301.7)	63.7 / 64.0	4.01 / 3.89	4.6 / 4.4	190–194	Pt.Et.	3280, 1660, 1630, 1590	
12	Cl	$NHCH_2CH_2Cl$	$C_{16}H_{11}Cl_2NO_2$ (320.2)	60.0 / 59.9	3.46 / 3.35	4.4 / 4.2	152–155	Pt.Et.	1660, 1630, 1590, 1240	153
13	see text	Cl	$C_{18}H_{15}ClN_2O_3$ (342.8)	63.1 / 63.2	4.41 / 4.69	8.2 / 7.9	216–218	EtOH	3440, 1630, 1320, 1240	

[+] Ad = 1-Adamantyl

The reaction between compound **2** and N-(2-hydroxyethyl)ethylenediamine gave compound **13**, while the open chain derivative **14** was not isolated.



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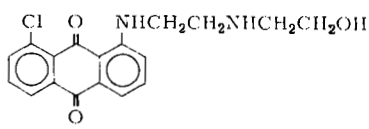

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Table 2: *^{1}H-NMR* [δ (ppm) in [a] $CDCl_3$, [b] d_6-DMSO] *of Compounds* **3–13** *and Mass Spectra of Compounds* **6–13**

3[a] 2.44 (6H, s, Me); 7.48 (2H, pseudo-q, arom., J = 8.1 Hz, J = 1.5 Hz); 7.85 (2H, pseudo-t, arom., J = 8.1 Hz); 8.33 (2H, pseudo-q, arom., J = 8.1 Hz, J = 1.5 Hz).

4[a] 1.33 (6H, t, Me); 2.75 (4H, q, CH_2); 7.45 (2H, pseudo-q, arom., J = 8.1 Hz, J = 1.5 Hz); 7.8 (2H, pseudo-t, arom., J = 8.1 Hz); 8.28 (2H, pseudo-q, arom., J = 8.1 Hz, J = 1.5 Hz).

5[a] 1.1 (6H, t, Me); 1.86 (4H, sex, CH_2); 2.65 (4H, t, CH_2); 7.41 (2H, pseudo-q, arom., J = 8.1 Hz, J = 1.5 Hz); 7.73 (2H, pseudo-q, arom., J = 8.1 Hz); 8.25 (2H, pseudo-q, arom., J = 8.1 Hz, J = 1.5 Hz).

6[a] 1.73 (12H, broad, Ad); 2.06 (18H, broad, Ad); 7.36 (2H, pseudo-q, arom., J = 8.1 Hz, J = 1.5 Hz); 7.76 (2H, pseudo-t, arom., J = 8.1 Hz); 8.28 (2H, pseudo-q, arom., J = 8.1 Hz, J = 1.5 Hz); m/e = 564 (6 %, M^+); 526 (62); 499 (8); 394 (100).

7[a] 3.45 (3H, s, Me); 7.33 (1H, m, arom.); 7.6–8.0 (4H, m, arom.); 8.38 (1H, pseudo-q, arom.); m/e = 318 (43 %, M^+); 240 (100); 212 (12); 211 (19).

8[b] 3.66 (6H, s, Me); 8.23 (2H, pseudo-q, arom., J = 7.5 Hz, J = 2.0 Hz); 8.4 (2H, pseudo-t, arom., J = 7.5 Hz); 8.6 (2H, pseudo-q, arom., J = 7.5 Hz, J = 2.0 Hz); m/e = 396 (30 %, M^+); 318 (91); 253 (50); 240 (100).

9[a] 1.28 (3H, t, Me); 2.7–3.2 (4H, m, CH_2CH_2); 4.23 (2H, q, CH_2); 7.23–7.93 (5H, m, arom.); 8.3 (1H, pseudo-q, arom., J = 8.1 Hz, J = 1.5 Hz); m/e = 368 (10 %, M^+); 318 (41); 236 (100).

10[b] 1.33 (6H, t, Me); 2.8–3.3 (8H, m, CH_2CH_2); 4.3 (4H, q, CH_2); 8.0 (2H, pseudo-q, arom., J = 8.1 Hz, J = 1.5 Hz); 8.38 (2H, pseudo-t, arom., J = 8.1 Hz); 8.6 (2H, pseudo-q, arom., J = 8.1 Hz, J = 1.5 Hz); m/e = 496 (26 %, M^+); 451 (63); 396 (23); 368 (39); 323 (100).

11[b] 3.5 (2H, m, CH_2); 3.8 (2H, m, CH_2); 5.1 (1H, t, OH); 7.4–8.3 (5H, m, arom.); 8.46 (1H, pseudo-q, arom.); 9.8 (1H, t, NH); m/e = 303 (9 %, M^+); 301 (28); 283 (14); 272 (56); 270 (100).

12[b] 3.9 (2H, m, CH_2); 4.0 (2H, m, CH_2); 7.5–8.2 (5H, m, arom.); 8.4 (1H, pseudo-q, arom.); 10.0 (1H, broad, NH); m/e = 321 (14 %, M^+); 319 (36); 284 (28); 276 (57); 270 (100).

13[b] 3.7 (8H, m, CH_2CH_2); 5.0 (1H, t, OH); 7.0 (1H, d, arom.); 7.7 (1H, d, arom.); 8.03 (2H, m, arom.); 8.46 (1H, pseudo-q, arom.); 10.43 (1H, broad, NH); m/e = 344 (15 %, M^+); 342 (35); 313 (27); 311 (64); 272 (43); 270 (100).

The cyclized form for compound **13** was assigned on the basis of the ^{1}H-NMR and mass spectra. The aromatic region of the ^{1}H-NMR spectrum shows only five protons and the relative signals (two

doublets at δ = 7.0 and δ = 7.7 ppm, a pseudo-quartet at δ = 8.46 and a signal deriving from the superimposition of a pseudo-triplet and a pseudo-quartet at δ = 8.03 ppm) are in agreement with the assigned structure. Furthermore the signal of the NH group at δ = 10.43 ppm is a broad singlet, while the signals relative to the NH groups for compounds **11, 12** are triplets. The mass spectrum of compound **13** (mw 342.78) clearly shows two peaks at m/e 344 and 342 with relative intensity corresponding to the natural abundance of the isotopic composition of chlorine.

Pharmacological Results

Compounds **3–13** were tested in mice bearing Ehrlich ascites tumor cells (see Exp. Part). % T/C is reported in Table 1 only for compounds showing significant activity. While it is not surprising that compound **12** showed antitumor activity, we wish to underline the behaviour of the succinic esters **9, 10**. The mechanism of action of these two derivatives is still under investigation but we think that their activity could be due to an aminolysis reaction: in fact compounds **9** and **10** completely react in 24 h with benzylamine in acetone at room temp. yielding compound **1** and the N-benzylamide **15** (see Exp. Part); in contrast, compounds **3–8** do not react under the same experimental conditions.

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Experimental Part

A) Chemistry

MP: uncorr.; Bakerflex plates (Silica-gel IB2-F) were used for *TLC. Column chromatography*: Kieselgel 60 (Merck) activated at 120 °C for 2 h, in the proportion of 30 g per gram of substance: unless otherwise stated, the eluent is petroleum ether (bp 60–80°)/acetone (80/20). *IR spectra*: (Nujol) Perkin-Elmer 298 spectrometer. *^{1}H-NMR-spectra*: Varian XL-100, TMS as int. stand. *MS*: Varian 112S.

Synthesis of compounds **3–6**

To a solution of 3 g (12.5 mmoles) of compound **1** in 50 ml pyridine was slowly added, with cooling ($t \leq 15°$) and stirring, 25 mmoles of the appropriate acyl chloride. The reaction mixture was then stirred for 3 h at room temp., poured onto ice and acidified with 6N-HCl. A quantitative amount of the ester was collected by filtration and crystallized (see Tables 1, 2).

Synthesis of compounds **7–10**

With the same procedure described above, mesyl chloride gave a mixture of **1** (25 %), **7** (40 %) and **8** (35 %), while ethyl succinyl chloride gave a mixture of **1** (10 %), **9** (40 %) and **10** (50 %) (densitometric TLC). Compounds **7–10** were isolated by column chromatography and crystallized (see Tables 1, 2). The yield of the diesters **8, 10** may be increased by heating the reaction mixture before pouring onto ice.

Aminolysis of compounds **9, 10** *with benzylamine*

Compound **9** (1 mmole) and benzylamine (1 mmole) were dissolved in 30 ml acetone and kept at room temp. for 24 h. The mixture was then evaporated to dryness and the residue separated by column chromatography (benzene): from the first yellow eluate, compound **1** was isolated in 85 % yield; the second colourless eluate contained the N-benzylamide **15**: an analytical pure sample was prepared by means of preparative TLC (SiO_2-benzene/acetone 95/5): yield 45 %; mp 47 °C from petroleum ether; ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.24 (3H, t, $CH_2\underline{CH_3}$); 2.64 (4H, m, CH_2CH_2); 4.20 (2H, q, $\underline{CH_2}CH_3$); 4.50 (2H, d, $\underline{CH_2}C_6H_5$); 6.4 (1H, broad, NH); 7.41 (5H, s, arom.). This compound proved identical to a sample obtained directly from the *Schotten-Baumann* reaction between benzylamine and ethyl succinyl chloride. Under the same experimental conditions, starting from compound **10** (1 mmole) and benzylamine (2 mmoles), compound **1** was isolated in 90 % yield and the N-benzylamide **15** in 55 % yield.

Synthesis of compounds **11, 12**

Compound **2** (2.8 g, 10 mmoles) was treated with 20 g (190 mmoles) diethanolamine and 20 ml pyridine; the reaction mixture was refluxed for 12 h, then it was poured onto ice and acidified with 6N-HCl: the red precipitate (80 %) was crystallized (**11**, see Tables 1, 2). Compound **11** was refluxed for 5 h with an excess of $SOCl_2$; after cooling, $SOCl_2$ was removed under reduced pressure and the residue was crystallized with a yield of 40 % (**12**, see Tables 1, 2).

Synthesis of compound **13**

Compound **2** (2.8 g, 10 mmoles) was treated with 4.16 g (40 mmoles) N-(2-hydroxyethyl)ethylenediamine and 30 ml pyridine; the reaction mixture was refluxed for 6 h, then it was poured onto ice, acidified with 6N-HCl and extracted with chloroform. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure; the residue was crystallized yielding 30 % of compound **13**. An analytical sample was prepared by submitting the residue to column chromatography (see Tables 1, 2).

B) Pharmacology

Eight female Swiss mice (average weight 21 ± 1 g) were implanted with 10^6 Ehrlich ascites tumor cells from donor mice. After 24 h the animals were treated i.p. with a single dose (200 mg/kg) of the compound under test **3–13** dissolved in DMSO: the amount of DMSO used was shown, in analogous experiments, not to affect tumor growth. If the dose proved toxic or active, the test was repeated at lower doses (100–50–25 mg/kg) with other groups of eight mice. Deaths were recorded for the 60-day period. The activities were measured as the ratio of the mean survival time of the test animals to that of the control animals (ten mice) expressed as a percentage (% T/C). Significant activity is achieved with an increased life span of 25 % (T/C ≥ 125).

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Reduktionen substituierter 3,7-Diaza- und 3-Thia-7-azabicyclo[3.3.1]nonan-9-one mit Natriumborhydrid

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Substituierte 3,7-Diaza- und 3-Thia-7-azabicyclo[3.3.1]nonan-9-ole werden durch Reduktion der entsprechenden Ketone mit Natriumborhydrid erhalten. Die Stereoselektivität der Reduktion ist in wäßrigem Dioxan sehr hoch, während in Methanol Epimerengemische entstehen. Die ^{1}H-NMR-Spektren der epimeren Alkohole werden vergleichend diskutiert.

Reductions of Substituted 3,7-Diaza- and 3-Thia-7-azabicyclo[3.3.1]nonan-9-ones with Sodium Borohydride

Substituted 3,7-diaza- and 3-thia-7-azabicyclo[3.3.1]nonan-9-oles are prepared by reduction of the corresponding ketones with sodium borohydride. The stereoselectivity of this reduction is very high in dioxane/water as solvent; in methanol, mixtures of epimers are obtained. The ^{1}H-NMR spectra of the epimeric alcohols are discussed.

1-Aza[1]- und 1-Thiacyclohexanon-3,5-dicarbonsäureester[2] lassen sich aufgrund der CH-Acidität in 3- und 5-Position mit einem primären Amin und Formaldehyd zu 3,7-Diaza- bzw. 3-Thia-7-azabicyclo[3.3.1]nonan-9-onen **1–4** aminomethylieren (Abb. 1). Die Konfiguration der Ausgangsprodukte ist bereits früher aufgeklärt worden[3a]; eine Sessel/Sessel-Konformation für die hier beschriebenen Bicyclononanone ist wahrscheinlich[3a,3b].

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