10-(ω-Carboxyacyl)-dithranol-Derivatives

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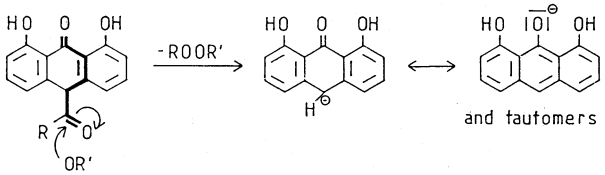
The title compounds are not available by hydrolysis of the pertinent esters on a preparative scale. Therefore, they were prepared by base catalyzed condensation of dicarboxylic acid dichlorides or succinic acid monobenzylesterchloride, respectively, with dithranol (**1**). Their IC_{50}-values for glucose-6-phosphate dehydrogenase are lower than that of dithranol (**1**), whilst 10-ethyldithranol (**6**) and the o-(ω-carboxyalkyl)-derivatives **8** and **9** are weaker inhibitors.

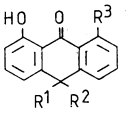
10-(ω-Carboxyacyl)-Derivate des Dithranols

Die Titelverbindungen sind nicht aus den entspr. Estern präparativ zugänglich, sondern wurden aus Dicarbonsäuredichloriden bzw. Bernsteinsäuremonobenzylesterchlorid und Dithranol (**1**) hergestellt. Die IC_{50}-Werte dieser Substanzen für Glucose-6-phosphat-Dehydrogenase sind kleiner als der von Dithranol (**1**), 10-Ethyldithranol (**6**) und die o-(ω-Carboxyalkyl)-Derivate **8** und **9** sind dagegen schwächere Inhibitoren.

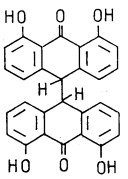
10-Acyl-derivatives of dithranol (**1**) have been introduced as antipsoriatic agents by *Mustakallio*[1], butantrone (**2b**) being the most effective compound of a series with 2 to 5 and with 14 C-atoms in the C-10 side chain[2]. According to *Krebs*[3] the C-10-acylated dithranol derivatives are considered to be pro-drugs on account of their phenylogous β-dicarbonyl moiety.



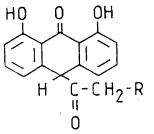
We have reported on the synthesis of 10-(ω-methoxycarbonyl)-acyl-dithranol derivatives (type **3**)[4]. One of these compounds has been described by *Rychener* et al.[5] as the ethoxy analogue in the meantime. Compounds **3** cannot be hydrolized to the corresponding 10-(ω-carboxyacyl)-derivatives **4** on a preparative scale[6]. We tried LiBr/pyridine; BBr_3/absol. CH_2Cl_2;$ClSi(CH_3)_3$/NaI/CH_3CN and NaI/$AlCl_3$/CH_3CN, but we obtained always dithranol (**1**) besides - in some cases - starting material **3**. These findings are corroborated by efforts of *Rychener* et al.[5] with compound **3a** (ethoxy), who found a peak in the HPLC-chromatogram which they considered to be acid **4a**, but they could neither isolate nor identify it. We have synthesized the carboxylic acid **4a** not via the corresponding esters but by acylation of **1** at C-10 making use of succinic acid monobenzylesterchloride (yielding **10**) with subsequent hydrogenolysis. For the synthesis of **4b** and **4c** we reacted the dichlorides of glutaric and adipic acid, respectively, with dithranol (**1**). With adipic acid dichloride we isolated the corresponding lactone **5b** as a side product. With succinylchloride only the lactone **5a** is formed[4]. The tlc of the crude material formed from **1** and glutarylchloride indicates traces of an analogous lactone which was not isolated. The retention time of acid **4a** (t = 4.55 min) under the HPLC experiment conditions reported



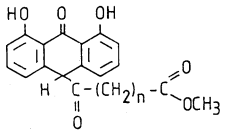
1: $R^1 = R^2 = H$; $R^3 = OH$
1a: $R^1 + R^2 = O$; $R^3 = OH$
6: $R^1 = C_2H_5$; $R^2 = H$; $R^3 = OH$
7: $R^1 = R^2 = R^3 = H$



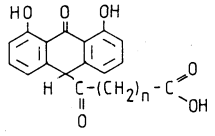
1b



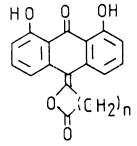
2a: R = H
2b: $R = C_2H_5$



3a: n = 2
3b: n = 3



4a: n = 2
4b: n = 3
4c: n = 4



5a: n = 2
5b: n = 4

**) Dedicated to Prof. Dr. E. Röder, Bonn, on the occasion of his 60th birthday.

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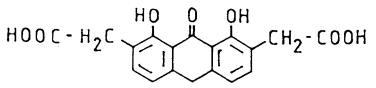

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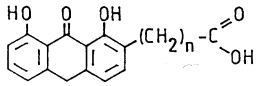
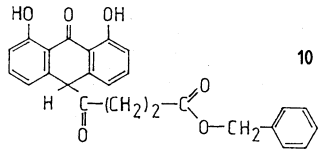


9a: n=1
9b: n=2



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by *Rychener* et al.[5] corroborate their assumption that the compound not isolated in their experiments (vide supra) is indeed **4a**.

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49, from Baker's yeast, G-6-PDH) is highly increased in psoriatic tissues[7]. So a lot of publications are concerned with the interaction of (potential) antipsoriatics with this enzyme in vitro (e.g. *Raab*[8], *Wiegrebe*[9], *Shroot*[10] ...). This kind of experiments is important because psoriasis is not known in animals and, therefore, there are analogous[11),12)] but no strict animal models available to test new compounds.

We tested 10-ethyl-dithranol (**6**)[13], representing a C-10-alkylated dithranol, 1-hydroxy-9-anthrone (**7**)[14], the minimal antipsoriatic structure according to *Krebs* and *Schaltegger*[15], C-2-(ω-carboxy-alkyl)-dithranol derivatives [**8**,**9**][4], C-10-acylated dithranol derivatives [**2**,**3**,**4**][16),4] and the lactones **5** without a "free" CH_2-moiety. We want to emphasize that these lactones are effective by themselves: after 30 min of incubation (cf. Experimental Part) about 75% of the lactone **5b** were determined unchanged, about 10% of chrysazin (**1a**) have arisen, but no significant quantity of the corresponding acid **4c** was detected by HPLC.

The IC_{50}-values of the compounds listed in table 1 were determined by graphic means from at least 7 points (fig. 1). For each concentration the mean value of three

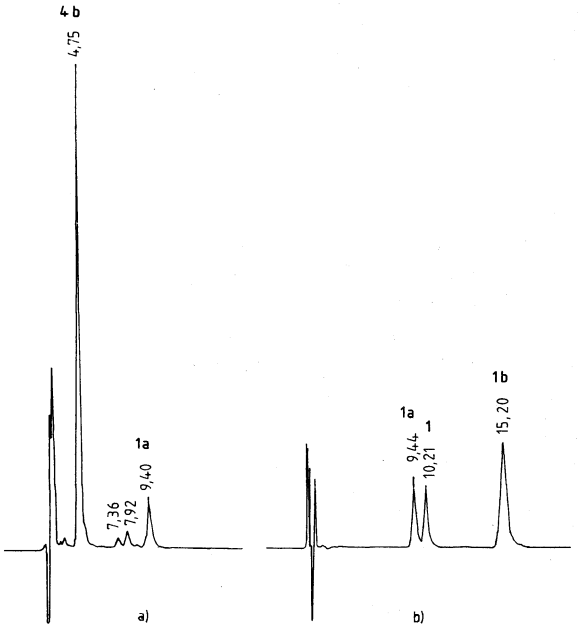

Fig.2: HPLC-separations

Table 1: IC_{50}-values of all tested compounds

compound	IC_{50}-value
8	$6.6 \cdot 10^{-4}$M
9	$3.8 \cdot 10^{-5}$M
6	$2.2 \cdot 10^{-5}$M
9b	$1.8 \cdot 10^{-5}$M
7	$9.4 \cdot 10^{-6}$M
3b	$9.2 \cdot 10^{-6}$M
1	$8.0 \cdot 10^{-6}$M
3a	$6.4 \cdot 10^{-6}$M
2a	$5.0 \cdot 10^{-6}$M
5a	$1.7 \cdot 10^{-6}$M
5b	$1.7 \cdot 10^{-6}$M
2b	$1.3 \cdot 10^{-6}$M
4c	$9.0 \cdot 10^{-7}$M
4a	$2.8 \cdot 10^{-7}$M
4b	$2.2 \cdot 10^{-7}$M

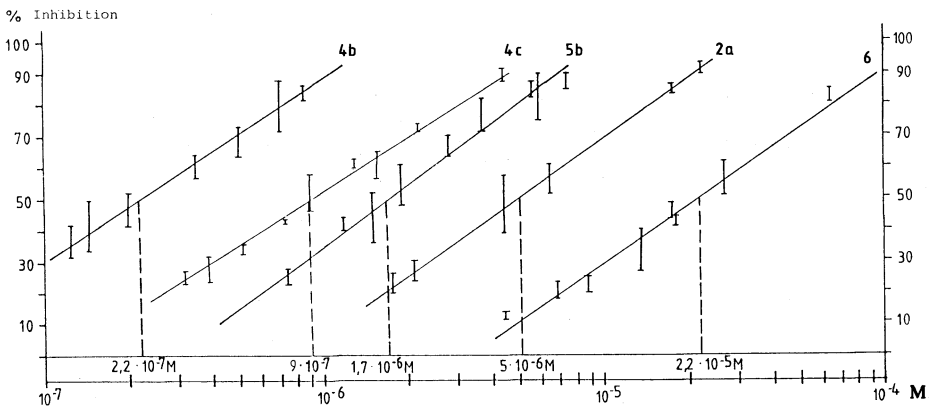

Fig.1: Inhibition of Glucose-6-phosphate Dehydrogenase; IC_{50} values of selected compounds

determinations was used. The inhibition rate is shown as % of the control (cf. Experimental Part).

According to *Rychener* et al.[5] dithranol (**1**) is a more potent inhibitor than his ester **3a** (ethyl instead of methyl). In our experiments the IC_{50}-values of **3a** and **3b** are of similar magnitude as that of **1** (table 1). On the other hand the free acids **4a-c** are far stronger inhibitors than dithranol. This holds true also for the lactones **5a,b** and for butantrone **2b**, so making *Krebs'* hypothesis of a pro-drug character[3] of C-10-acylated dithranol derivatives disputable. Moreover, our HPLC experiments of the incubation tests indicate that there is neither dithranol (**1**), nor its degradation product **1b** to be seen after 30 min of incubation of all the C-10-acylated dithranol derivatives tested (fig. 2a)). Dithranol (**1**), however, is degraded nearly perfectly, bianthron (**1b**, about 60%) and chrysazin (**1a**, about 20%) were found as degradation products (fig. 2b)). The mechanism for the formation of minor amounts of chrysazin (**1a**) from the lactone **5b** and the acid **4b** (fig.2) is unknown.

These experiments indicate that compounds **3**, **4**, and **5** are effective by themselves in this test. If dithranol (**1**) formed by hydrolysis of **3-5**, respectively, were the active principle, the efficacy of the lactones **5** and acids **4** should not exceed that of **1**.

Experimental Part

Devices: Mp.: (uncorr.) apparatus according to Dr. *Tottoli* (Büchi).- UV-spectra: Shimadzu 210; 1 cm cells.- IR-spectra in KBr: Beckman Acculab III.- ^{1}H-NMR-spectra: Varian EM 390, $CDCl_3$, 35°C, TMS as int. stand.- MS: Varian MAT CH5, 70 eV.- NI-FAB-MS (glycerol/DMSO 1:1; Xe) Varian MAT 311A.- HPLC: pump: Kontron 420; UV-detector: Kontron Uvikon 735 LC; integrator: Merck-Hitachi D-2000; injection system: Rheodyn.- All the reactions were performed under N_2 and light protection.

1,8-Dihydroxy-10-(3'-benzyloxycarbonyl-1'-oxopropyl)-9(10H)anthracenone (**10**)

The suspension of 2.26 g **1** (10 mmol) and 3.0 g (13.25 mmol) succinic acid benzylester-chloride[17] in 80 ml of absol. toluene and 1.0 g (13 mmol) of dry pyridine is refluxed for 6 h. The resulting orange solution is evaporated i.vac., the residue, dissolved in a small volume of CH_2Cl_2, is separated from **1** (1 g, 44%) by CC (SiO_2/CH_2Cl_2): 1.4 g **10** (60%, related to reacted **1**), yellow crystals, m.p. 125°C.- $C_{25}H_{20}O_6$ (416.4).- Calc. C 72.1 H 4.84 Found C 71.9 H 4.80.- UV (MeOH): λ max (log ε) = 359 (4.02), 279 (3.97), 261 (4.18), 208 nm (4.44).- IR: 1750 (COOR); 1720 (C=O); 1640; 1615; 1605 cm^{-1} (C=O···HO).- ^{1}H-NMR: δ (ppm)= 12.20 (s; 2H, OH), 7.60-6.83 (m; 11H aromat.), 5.22 (s; 1H at C-10), 5.0 (s; 2H; -O-CH_2-Ph), 2.39 (s; 4H; -CH_2-CH_2-).

4-(1,8-Dihydroxy-9(10H)-anthracenon-10-yl)-4-oxo-butyric acid (**4a**)

600 mg **10** are dissolved in 30 ml of absol. THF. After addition of 70 mg Pd-C (5% Pd) the solution is stirred until the theoretical amount of H_2 has been absorbed (24 h, tlc-control). Having sucked off the catalyst the solution is evaporated i.vac. Recrystallisation from MeOH/H_2O leads to 350 mg (75%) **4a**, yellow crystals, m.p. 165°C.- $C_{18}H_{14}O_6$ (326.3).- Calc. C 66.3 H 4.32 Found C 66.3 H 4.63.- UV (MeOH): λ max (log ε) = 357 (3.96), 285 (3.88), 257 (3.94), 203 nm (4.37).- IR: 3060-2600 (COOH); 1720 (C=O); 1640; 1615; 1605 cm^{-1} (C=O···HO).- ^{1}H-NMR: δ (ppm) = 11.98 (s; 2H, OH), 7.75-6.98 (m; 6H aromat.), 5.70 (s; 1H at C-10), 2.90 (t; J = 7Hz; 2H; CH_2 at C-2), 2.31 (t; J = 7Hz; 2H, CH_2 at C-3).- NI-FAB-MS: m/z = 325 [(M-H)$^-$; 3.4%], 225 [(M-H)$^-$ - $C_4H_5O_3$; 100%].- PI-FAB-MS: m/z = 327 (MH^+; 54%), 227 (MH^+ - $C_4H_5O_3$; 100%).

5-(1,8-Dihydroxy-9(10H)-anthracenon-10-yl)-5-oxo-pentylic acid (**4b**)

2.26 g **1** (10 mmol) and 4.0 g (23.7 mmol) glutarylchloride[18] are suspended in 70 ml of absol. toluene and 1.0 g (13 mmol) of dry pyridine. The suspension is refluxed for 2 h (tlc-control). The resulting solution is evaporated i.vac. The residue is dissolved in ether and shaken with saturated $NaHCO_3$-solution changing the colour of the alkaline solution to red. Acidifying the aqueous layer with acetic acid yields a yellow coloured solution which is extracted with ether. The combined ether layers are dried over Na_2SO_4 and evaporated i.vac. Recrystallisation from MeOH/H_2O leads to 2.21 g (65%) **4b**, yellow crystals, m.p. 125°C.- $C_{19}H_{16}O_6$ (340.3).- Calc. C 67.1 H 4.74 Found C 66.9 H 4.74.- UV (MeOH): λ max (log ε) = 357 (3.99), 281 (3.99), 260 (4.07), 215 nm (4.20).- IR: 3040-2600 (COOH); 1720 (C=O); 1705 (COOH); 1635; 1615; 1605 cm^{-1} (C=O···HO).- ^{1}H-NMR ([D_6]DMSO/$CDCl_3$ 1:1): δ (ppm) = 12.0 (s; 2H; OH), 7.68-6.9 (m; 6H aromat.), 5.4 (s; 1H at C-10), 2.4 (t; J = 7Hz; 2H; CH_2 at C-2), 2.0 (t; J = 7Hz; CH_2 at C-4), 1.58 (quint.; J = 7Hz; CH_2 at C-3).- NI-FAB-MS: m/z = 339 [(M-H)$^-$; 58%], 225 [(M-H)$^-$ - $C_5H_7O_3$; 100%].- PI-FAB-MS: m/z = 341 (MH^+; 36%), 227 (MH^+ - $C_5H_7O_3$; 100%).

6-(1,8-Dihydroxy-9(10H)-anthracenon-10-yl)-6-oxo-hexylic acid (**4c**)

4c is prepared analogously to **4b** from 2.26 g **1** (10 mmol) and 2.77 g (15 mmol) adipic acid dichloride[19]: 360 mg (30%), yellow crystals, m.p. 128°C.- $C_{20}H_{18}O_6$ (354.4).- Calc. 67.8 H 5.12 Found C 67.8 H 5.18.- UV (MeOH): λ max (log ε) = 358 (3.99), 260 (4.18), 209 nm (4.31).- IR: 3420 (OH); 3040-2800 (COOH); 1720 (C=O); 1705 (COOH); 1640; 1620; 1610 cm^{-1}.- ^{1}H-NMR: δ (ppm) = 12.15 (s; 2H, OH, br.), 10.5-9.1 (s; 1H; COOH, br.), 7.6-6.78 (m; 6H aromat.), 5.19 (s; 1H at C-10), 2.18-1.7 (m; 4H; CH_2 at C-2 and at C-5), 1.45-1.12 (m; 4H; CH_2 at C-3 and at C-4).- NI-FAB-MS: m/z = 353 [(M-H)$^-$; 14%], 225 [(M-H)$^-$ - $C_6H_9O_3$; 100%].- PI-FAB-MS: m/z = 355 (MH^+; 21%), 227 (MH^+ - $C_6H_9O_3$; 100%).

1,8-Dihydroxy-10-(7'-oxepanyliden-2'-on)-9(10H)-anthracenone (**5b**)

Working up the ether layer of **4c** by CC (SiO_2/CH_2Cl_2) yields 510 mg **1** (23%) and the lactone **5b**: 60 mg (1.8%), yellow crystals, m.p. 188-190°C.- $C_{20}H_{16}O_5$ (336.3).- Calc. C 71.4 H 4.79 Found C 71.4 H 4.83.- UV (MeOH): λ max (log ε) = 383 (4.12), 299 (4.01), 228 (4.54), 205 nm (4.38).- IR: 1765 (ε-lactone); 1630; 1605 cm^{-1} (C=O···HO).- ^{1}H-NMR: δ (ppm) = 12.11 (s; 1H; OH), 12.05 (s; 1H; OH), 7.6-6.9 (m; 6H aromat.), 2.92-2.51 (m; 4H; CH_2 at C-2' and at C-5'), 2.18-1.82 (m; 4H; CH_2 at C-3' and at C-4').- MS: m/z = 336 ($M^{+\cdot}$; 100%), 318 ($M^{+\cdot}$ - H_2O; 62), 290 (72), 252 ($M^{+\cdot}$ - $(CH_2)_4CO$; 84), 226 (100), 224 (67).

Inhibition of G-6-PDH

Solutions: For all the solutions bidistilled water is used. G-6-P-solution: 130 mg G-6-P-di-Na (Sigma) are dissolved in 10 ml water.- $NADP^+$-solution: 100 mg β-$NADP^+$-mono-Na (Sigma) are dissolved in 5 ml of 1% $NaHCO_3$-solution.- Buffer: Ringer solution: 8.0 g NaCl, 0.2 g KCl, 1.0 g $NaHCO_3$, 0.2 g $CaCl_2$ and 0.1 g $MgCl_2$ are dissolved in 900 ml of water. PH is adjusted to pH 7.5 with HCl and water is added to 1000.0 ml.- Enzyme-dilution: G-6-PDH (Sigma, type VII, from Baker's yeast) is used in a dilution of 1:1000 with buffer.- Test-solution: The concentration of the stock solution is about $4\cdot10^{-3}$M (1 mg/ml of substance to be tested, dissolved in acetone, p.a. Merck). Addition of 20 µl of the stock solution to the incubation volume leads to a concentration of $1.75\cdot10^{-5}$M in this solution (5 ml). The variety of concentrations is prepared by diluting different amounts

of stock solution with acetone. Incubation preparation (5 ml): For each value 4.48 ml buffer, 0.5 ml enzyme dilution and 20 µl test-solution of the required concentration are shaken in a water bath for 30 min at 37°C under light protection. The corresponding control value (buffer - enzyme dilution - acetone), measured analogously, is determined every h because the activity of the enzyme decreases steadily. All solutions are freshly prepared just before measurement. For each concentration three determinations are made.

Determination: wavelength 340 nm, temp. 25°C, volume 3.0 ml, 1 cm cuvettes.

buffer	2.40 ml
incubation volume	0.50 ml
$NADP^+$-solution	0.05 ml
mix	
G-6-P-solution	0.05 ml

After mixing the extinction is measured every min during 5 min. The inhibition is expressed in % of the control.

HPLC-conditions

Column: Nucleosil 100, RP 18, 7 µm, 280 x 4 mm Kontron.- mobile phase: MeOH/H_2O/acetic acid (85:15:0.1).- flow: 1 ml/min.- pressure: 106 bar.- detection: 254 nm.- injection volume: 20 µl.

Determination of the retention time of each substance: about $1 \cdot 10^{-5}$M solutions in methanol are prepared and 20 µl are injected.

Retention time (min): **4a** (4.55), **4b** (4.75), **4c** (5.25), **3a** (5.87), **3b** (6.4), **5b** (8.24), **2b** (8.84), **1a** (9.44), **1** (10.21), **1b** (15.2).

In order to scrutinize the stability of these substances during 30 min of incubation (pH 7.5, water bath, light protection, 37°C), we examined these preparations by HPLC; especially we looked for dithranol (**1**): after 30 min the incubation volume (5 ml) is diluted with 10 ml of water and purified by a *Baker*-column (C-18, 10 SPE™). Elution of the substance with 3 ml MeOH; HPLC-injection. The recovery rate was determined for **3a** and **4b** and found to be 90 and 105%, respectively.

Results of HPLC

After 30 min of incubation 70-85% of the substances **2**, **3**, **4**, and **5** are found; dithranol (**1**) was never detected, but there are small amounts of chrysazin (**1a**, 5-20%). Dithranol (**1**) is not stable during this incubation (vide supra).

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