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New Biologically Active Rubiginones from *Streptomyces* sp.†

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Four new polyketides, named rubiginone D_2 (**2**), 4-*O*-acetyl-rubiginone D_2 (**3**), rubiginone H (**6**) and rubiginone I (**7**) were isolated from the cultures of *Streptomyces* sp. (strain Gö N1/5). Their structures were established by a detailed spectroscopic analysis. The absolute configuration of **3** was determined by derivatization with chiral acids (Helmchen's method). The rubiginones inhibit the growth of some Gram-positive bacteria and are cytostatically active against different tumor cell lines.

Angucyclines represent a rapidly enlarging family of antibiotics[2]. The chemical structures of these secondary metabolites consist of an angular tetracyclic benz[a]anthracene core connected with a variable number of hydrolyzable sugar moieties[3]. The aglyca of the angucyclines are designated as angucyclinones. The biosynthesis of their benz[a]anthracene core is performed by folding a hypothetical decaketide precursor which is built up by a polyketide synthase of the iterative type II from acetate/malonate units. The structural modification of the benz[a]anthracene backbone by post-polyketide working enzymes leads to structures which differ in their substitution pattern or their grade of aromaticity (especially of ring A and B). As a consequence of the structural modifications during the late biosynthesis angucyclines and angucyclinones show a wide range of biological activities.

In the course of our chemical screening[4] we discovered *Streptomyces* sp. (strain Gö N1/5), a talented producer of new angucyclinones. In this paper we describe the fermentation and isolation procedures leading to the new secondary metabolites. In addition to the elucidation of their chemical structure some data of their biological activity are also reported.

Fermentation and Isolation

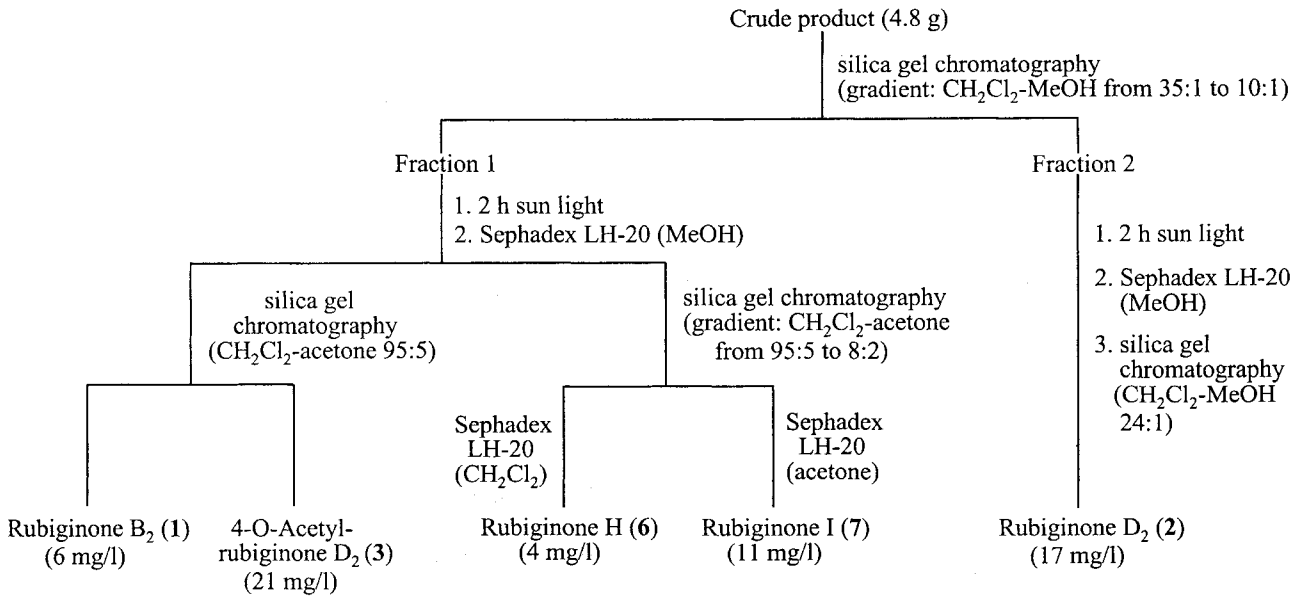
Streptomyces sp. (strain Gö N1/5), which was isolated from a soil sample collected near Hobas (Namibia), was cultivated in shaking flasks, using soybean meal/mannitol medium with Amberlite® XAD-2 as supplement for 72 hours at 28°C.

The concentrated methanolic extract of the mycelium was chromatographed on a silica gel column with CH_2Cl_2-MeOH gradient elution. Two collected fractions were of special interest due to their intensive yellow colour and their high sensitivity to light. In the presence of sunlight the colour of these fractions changed rapidly from yellow to red. To simplify the purification procedure of the photosensitive fractions, they were exposed to sunlight in the presence of atmospheric oxygen for 2 hours. Further chromatographic steps resulted in the isolation of five compounds (Scheme 1). Besides the known rubiginone B_2 (**1**)[5,6], we found three new angucyclinones and one new phthalide derivative.

The yields of the single metabolites are highly dependent on the fermentation conditions. The cultivation of strain Gö N1/5 in absence of Amberlite® XAD-2, yielded 2.8 mg/liter rubiginone B_2 (**1**) and 26 mg/liter rubiginone D_2 (**2**), but only traces of the other metabolites. A change of the

† Art. No. 40 on secondary metabolites by chemical screening. Art. No. 39: see ref. 1.

Scheme 1. Isolation and purification of the rubiginones.



medium from soybean meal/mannitol/XAD-2 to a glycerol/casein peptone medium also had considerable effects. It caused an increase of the yield of 4-*O*-acetyl-rubiginone D_2 (**3**) from 21 to 69 mg/liter and the production of additional metabolites like rubiginone A_2[5] (fujianmycin B[7]) and rubiginone E[8].

Physico-chemical Properties

The pure metabolites are optically active, detectable on TLC-plates with UV-light at 254 nm and when treated with anisaldehyde-H_2SO_4 show clearly recognizable colour reactions after heating (Table 1). They exhibit a good solubility in CH_2Cl_2, a weaker solubility in MeOH or acetone and are insoluble in *n*-pentane or water. Other physico-chemical data are given in the experimental part. Due to the mass-spectrometric and NMR spectroscopic investigations it could be confirmed that these five compounds are structurally related.

Structure Elucidation

According to the information obtained from EI-MS (m/z=320 [M^+]) and from NMR spectroscopy (the ^1H NMR spectrum shows signals of two methyl groups, two methylene groups, one aliphatic methine proton and five aromatic protons) the yellow compound with Rf 0.65 (Table 1) was identified as the already known rubiginone B_2[5] (X-14881 C[6]) (**1**).

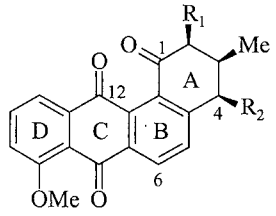
Table 1. Rf values and colour reactions of the isolated compounds.

A: $CHCl_3$ - MeOH (9 : 1), B: Acetone - cyclohexane (2 : 1).

Compound	A	B	Anisaldehyde-H_2SO_4
1	0.65	0.52	Violet Red
2	0.33	0.34	Dark Red
3	0.62	0.46	Violet Red
6	0.60	0.47	Blue
7	0.47	0.43	Blue Grey

The ^1H and ^{13}C NMR spectra of a second yellow compound exhibit 16 proton and 20 carbon signals. The APT spectrum indicates the presence of one *C*- and one *O*-methyl group, one aliphatic and two *O*-substituted methine groups, five aromatic methine groups, three carbonyl groups and seven other quaternary sp^2 carbon atoms. The molecular formula $C_{20}H_{16}O_6$ was deduced from a DCI mass spectrum (m/z=370 [$M+NH_4^+$]) in connection with an elemental analysis. The core structure of this metabolite is like rubiginone B_2 (**1**) a substituted 1,2,3,4-tetrahydro-3-methyl-8-methoxy-benz[a]anthraquinone, infered from its

Fig. 1. Structural formulae of rubiginone-type compounds.



	R_1	R_2
1	H	H
2	OH	OH
3	OH	OAc
4	(*S*)-COCH(Ph)Et	OAc
5	(*R*)-COCH(Ph)Et	OAc

UV spectrum, which is nearly identical to that of **1**. The substitution pattern of this compound was determined by means of 2D NMR spectroscopy using ^{1}H-^{1}H COSY, HETCOR and COLOC sequences (Figure 3). The values of the $^3J_{H,H}$ coupling constants between the three methine protons in ring A ($^3J_{2\text{-H},3\text{-H}}$=5.5 Hz, $^3J_{3\text{-H},4\text{-H}}$=3.0 Hz) indicate only axial-equatorial and/or equatorial-equatorial positions. Considering the stereochemical relations and ^{1}H,^{1}H-coupling constants of rubiginone B_2 (**1**)[5], rubiginone A_2[7,9] and SNA-8073-B[10] structure **2** could be established. According to the published metabolites of this type[5] we named the novel compound rubiginone D_2 (**2**).

The third compound which was isolated is very similar to **2**, but more lipophilic (Table 1). Its NMR spectra show an additional *O*-acetyl group (δ_H=2.09, δ_C=21.1 and 169.9) and a significant downfield shift of 4-H ($\Delta\delta$=1.19 ppm) verifying the position of the acetylation, which was confirmed by a HMBC experiment. All other signals coincide with those of **2** indicating 18 hydrogen and 22 carbon atoms (Table 2). In accordance with this the DCI-MS gave peaks suggesting a molecular weight of 394, compatible with the expected molecular formula $C_{22}H_{18}O_7$.

The application of Helmchen's method for chiral secondary alcohols[11] *via* esterification of 4-*O*-acetyl-rubiginone D_2 (**3**) with 2-(*S*)- and 2-(*R*)-phenylbutyric acid and ^{1}H NMR analysis of the isolated diastereomeric esters **4** and **5** (their δ values of 3-H and 3-CH_3 show a difference of $\Delta\delta_{S-R}$=0.08, respectively 0.18 ppm) resulted in the *S*-configuration of the centre of chirality at C-2. Therefore rubiginone D_2 (**2**) and 4-*O*-acetyl-rubiginone D_2 (**3**) should have the (2*S*,3*S*,4*R*) configuration.

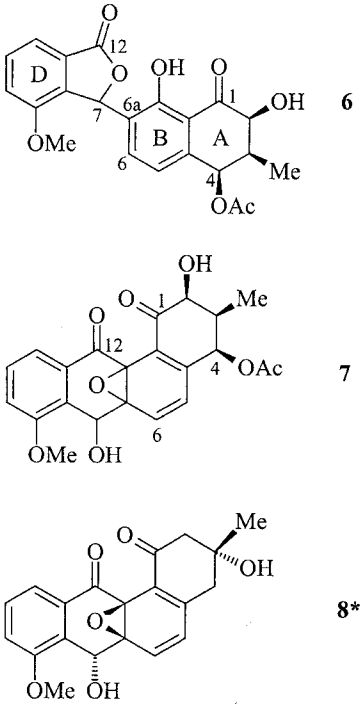
Table 2. ^{13}C NMR data of rubiginone D_2 (**2**), 4-*O*-acetyl-rubiginone D_2 (**3**), rubiginone H (**6**) and rubiginone I (**7**).

	2	**3**	**6**	**7**
C-atom	δ_C[a]	δ_C[b]	δ_C[b]	δ_C[b]
C-1	199.5	198.4	203.6	195.9
C-2	73.3	73.3[c]	72.0	71.9
C-3	44.8	42.6	39.6	41.2
3-CH_3	10.7	9.9	10.5	10.0
C-4	72.7	73.2[c]	73.0	72.6
C-4a	148.2	143.8	139.0	143.2
C-5	134.6	135.4[d]	121.9	130.8
C-6	131.2	131.6	136.6	134.1
C-6a	136.0	136.7	125.0	66.2
C-7	180.8	180.6	75.6	63.1
C-7a	120.1	120.4	128.4	127.1
C-8	159.8	159.9	154.4	156.6
8-OCH_3	56.4	56.5	55.7	55.9
C-9	117.5	117.4	115.4	115.2
C-10	135.7	135.7[d]	131.5	130.5
C-11	119.6	119.6	117.3	119.1
C-11a	137.1	137.3	135.7	132.4
C-12	183.7	183.6	170.3	191.9
C-12a	134.9	135.6	160.5	60.7
C-12b	132.1	133.0	114.3	128.5
4-OCO$\underline{C}$H$_3$		21.1	21.2	21.0
4-O$\underline{C}$OCH$_3$		169.9	169.9	170.4

[a] 75.5 MHz, $CDCl_3$; [b] 125.7 MHz, $CDCl_3$; [c,d] Assignment may be interchanged.

The molecular formula of a fourth compound was established as $C_{22}H_{20}O_8$ by HREI-MS (*m/z*=412.1158 [M^+]), it differs only in one molecule water from **3**, but the NMR data show that the core structure must be changed. One *O*-methyl, one *C*-methyl, one *O*-acetyl group and a signal pattern which is typical of ring A and D in **3** are present. Missing is the quinone moiety connecting ring B and D in **3**. Instead of a quinone carbonyl carbon atom there is a further ester linkage (δ_C=170.3), which must be part of a γ-lactone in accordance with the IR absorption at $\tilde{\nu}$=1770 cm^{-1}. An additional phenolic hydroxy group (δ_H=11.95), hydrogen bonded to the carbonyl group (δ_C=203.6) of ring A, and a highly conjugated methine group (δ_H=6.77, δ_C=75.6) also characterizes this compound. Detailed analysis of HSQC- and HMBC experiments (Figure 3) led to structure **6**, which contains a phthalide moiety. The absolute configurations of the centres of chirality at C-2, C-3 and C-4 were postulated analogously to **3**, the configuration of C-7 is still unknown.

Fig. 2. Structural formulae of rubiginone H (**6**), rubiginone I (**7**) and angucyclinone D (**8**)[14].



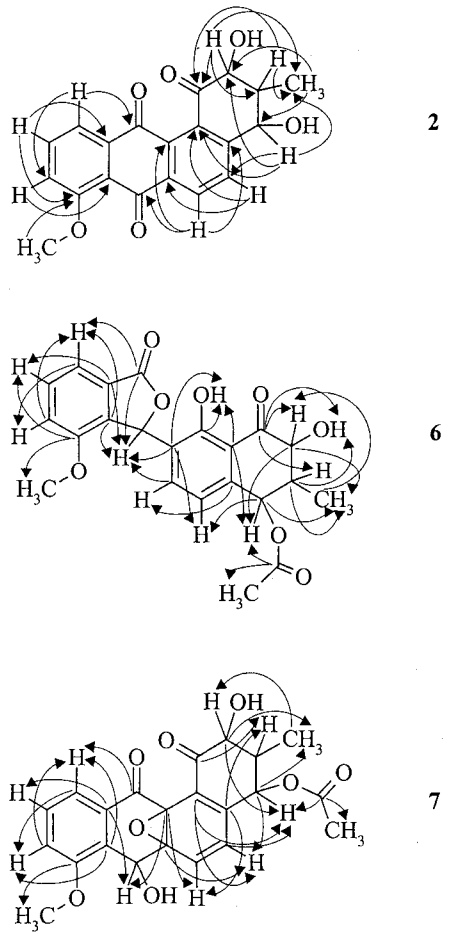
* Stereochemistry of the entired molecule is relative.

The compound with Rf 0.47 ($CHCl_3$-MeOH, 9:1) is isomeric to **6**, seen by its molecular ion at m/z=412 (EI-MS) and the molecular formula $C_{22}H_{20}O_8$ confirmed by HREI-MS. The NMR data resemble those of **3** more than those of the phthalide **6**. Compared with **3**, one quinone carbonyl group is reduced to a secondary alcohol (δ_H=2.45 and 5.92, δ_C=63.1) and two aromatic carbon atoms are converted to oxygen-substituted quarternary carbon atoms (δ_C=60.7 and 66.2), typical of an epoxide structure[12,13]. The positions of these structure elements were deduced from a HMBC experiment (Figure 3) and in comparison with the structurally related angucyclinone D (**8**)[2,14]. The absolute configurations of the centres of chirality of rubiginone I (**7**) at C-2, C-3 and C-4 were postulated following 4-*O*-acetyl-rubiginone D_2 (**3**).

Biological Activities

Against the four human cancer cell lines HMO2 (stomach adenocarcinoma), Kato III (colon carcinoma), HEP G2 (hepatocellular carcinoma) and MCF 7 (breast adenocarcinoma) rubiginone D_2 (**2**) and its 4-*O*-acetyl derivative **3** showed a significant growth inhibition (Table 3). The GI_{50}- and TGI-values of these two compounds are comparable to those of cisplatin. The activity of rubiginone B_2 (**1**) in the same test was obviously lower, but it was found out recently that **1** potentiates the cytotoxicity of vincristine against vincristine-resistant tumor cell lines[5].

Fig. 3. Important $^nJ_{C,H}$ long range couplings observed in **2** (by COLOC pulse sequences) and in **6** and **7** (by HMBC pulse sequences) at 300 MHz.



The antimicrobial activities of the isolated rubiginones determined in agar plate diffusion assays against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* are summarized in Table 4. None of the tested metabolites inhibited the growth of *Candida albicans*.

Discussion

Rubiginone D_2 (**2**), its 4-*O*-acetyl derivative **3** and rubiginone I (**7**) are angucyclinones of the tetrangomycin-

Table 3. Cytostatic activity against different tumor cell lines (GI_{50} and TGI values in μmol/liter)[15,16].

	HMO2		Kato III		HEP G2		MCF7	
Compound	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI
1	0.95	> 50	1.5	> 50	1.0	> 50	12	> 50
2	0.1	50	0.7	1.0	< 0.1	3.0	7.5	16
3	1.5	3.4	n. d.	n. d.	2.8	7.0	2.7	12.0
7	0.1	50	0.8	50	< 0.1	0.5	1.8	> 50
Cisplatin	0.17	1.5	2.4	> 50	0.5	5.0	0.1	10

GI_{50}=concentration, which results in a 50%-inhibition of the cell growth.
TGI=concentration, which results in a complete inhibition of the cell growth.

Table 4. Diameter of inhibition zone (mm) caused by 64 μg of **1**, **2**, **3**, **6** and **7** in the agar plate diffusion assay (the diameter of the used assay discs was 9 mm).

test organism	**1**	**2**	**3**	**6**	**7**
B. subtilis	20	0	19	14	31
S. aureus	16	16	22	13	23
E. coli	20	22*	16	13	33

* Incomplete inhibition zone.

type containing a highly oxygenated ring A. The presence of a hydroxy group at C-2 is unusual for angucyclinones, only few examples are known (*e.g.* rhodonocardin A and B[17], sakyomycin A, B and D[18], PD 116,779[19]). In contrast to **1** and **2** the three other rubiginones are monoacetates. Amazingly, only some rearranged angucylinones, *e.g.* momofulvenone B[20] or kinamycin E[21], exhibit an acetoxy substituent at C-4.

Rubiginone H (**6**) has a similar oxygen-substitution pattern like **7**, but it consists of a substituted phthalide which is connected with a tetralone moiety. Biosynthetic considerations led to the conclusion that the novel carbon skeleton of **6** also derives from a benz[a]anthracene intermediate. Possible precursors of rubiginone H (**6**) are 4-*O*-acetyl-rubiginone D_2 (**3**) or rubiginone I (**7**). The conversion of **3** into **6** requires an oxidative and regioselective C–C bond cleavage at C-12/C-12a by means of a "Baeyer-Villiger-enzyme", followed by hydrolysis, reduction of the formed diarylketone and cyclisation of the γ-hydroxy acid to its corresponding five-membered ring lactone. A similar mechanism was proposed for the transformation of ochromycinone to emycin E by ROHR *et al.*[22]. Alternatively, an enzymatic- or acid-catalysed fragmentation of **7** could lead to the formation of rubiginone H (**6**) in one step.

The antibacterial activities of the four new compounds are similar to those of rubiginone B_2 (**1**), but, however, the higher oxygenated benz[a]anthracene derivatives **2**, **3** and **7** inhibit the growth of the four examined tumor cell lines significantly better than **1**. In this context it seems to be worth mentioning that the antibiotic-683, which is structurally related to rubiginone I (**7**), also shows antibacterial and anticancer activities[2,23].

Being familiar with the chemical structures of the isolated compounds, we are now able to explain the observed photosensitivity of some enriched fractions. As it has been described recently[24~26], angucyclinones with a secondary hydroxy group at C-1, undergo a photoenolization (Norrish type II reaktion) in CH_2Cl_2 in the presence of light. In the case of rubiginone B_1 the reddish enol would be oxidized to rubiginone B_2 (**1**)[9]. Thus, we assume that **2**, **3** and **7** are products of a similar photooxidation. The primary fermentation products should be the corresponding alcohols, which are reduced at C-1.

Experimental

General

MP's were determined on a Reichert hot-stage microscope and are not corrected. NMR spectra were measured with Varian Unity 300 (300 MHz), Bruker AMX 300 (300 MHz) and Varian Inova 500 (500 MHz) instruments. Chemical shifts are expressed in δ values with solvents as internal standards. The mass spectra were taken

by Finnigan MAT 95 (EI-MS: 70 eV, high resolution with perfluorkerosine as internal standard; DCI-MS: 200 eV, NH_3 as reactant gas). IR spectra in pressed KBr discs were recorded on a Perkin Elmer FT IR-1600 spectrometer and the UV spectra on a Kontron Uvikon 860 spectrophotometer. Optical rotation values were recorded with a Perkin Elmer 241 polarimeter and CD spectra with a Jasco J 500 A spectrometer. The elemental analysis was measured by Mikroanalytisches Labor der Universität Göttingen. TLC was carried out on silica gel 60 F_{254} plates (Merck, 0.25 mm) and column chromatography on silica gel (Macherey & Nagel, <0.08 mm) or Sephadex LH-20 (Pharmacia). Rf values were determined on 20×20 cm plates, the evaluation length was 10 cm. Compounds were viewed under UV lamp at 254 nm and sprayed with anisaldehyde-H_2SO_4 followed by heating.

Fermentation and Isolation

Streptomyces sp. (strain Gö N1/5) was maintained as a stock culture on agar plates containing degreased soybean meal 2%, mannitol 2% and agar 2% stored at 6°C. Fermentations were carried out in 1000 ml Erlenmeyer flasks with three baffles. Caps of foamed plastic material were used as closures. Each flask was filled with 150 ml of medium composed of soybean meal 2% and mannitol 2% in deionized water (adjusted to pH 7.0 before sterilization), sterilized 30 minutes at 121°C and then inoculated at room temperature with a 6 cm^2 piece of agar from 7-day-old cultures, which were grown at 28°C. The submerged cultures were cultivated on a rotary shaker (250 rpm) at 28°C. After 30 hours 5 g of sterilized Amberlite® XAD-2 was added to each flask and the cultures were shaked for additional 42 hours. The harvested culture broth (1.8 liter, pH=8.4) was separated into mycelium and culture filtrate by filtration. The resin containing mycelium was extracted three times with 400 ml of MeOH and the extract was concentrated to dryness *in vacuo*. The resulting crude product was exposed to light and purified as illustrated in Scheme 1.

Rubiginone D_2 (**2**)

MP 182°C; $[\alpha]_D^{22}$ +151° (*c* 0.19, MeOH); UV (MeOH) λ_{max} nm (lg ε) 263 (4.49), 376 (3.67); IR ν_{max} (KBr) cm^{-1} 3526, 3454, 1697, 1667, 1587, 1296, 1271, 1225, 1058; CD (MeOH) λ_{extr} nm ([θ]) 234 (+2.21×10^4), 271 (+3.60×10^4), 293 (−2.48×10^3), 325 (+9.33×10^3), 401 (−4.25×10^3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (d, *J*=7.2 Hz, 3H, 3-CH_3), 2.87 (qdd, *J*=7.2, 5.5, 3.4 Hz, 1H, 3-H), 3.64 (br, 2H, 2-OH, 4-OH), 4.01 (s, 3H, 8-OCH_3), 4.83 (d, *J*=3.4 Hz, 1H, 4-H), 5.33 (d, *J*=5.5 Hz, 1H, 2-H), 7.28 (dd, *J*=7.5, 2.2 Hz, 1H, 9-H), 7.63~7.72 (m, 2H, 10-H, 11-H), 7.76 (d, *J*=8.0 Hz, 1H, 5-H), 8.26 (d, *J*=8.0 Hz, 1H, 6-H); ^{13}C NMR (see Table 1); DCI-MS *m/z* (%) 370 (30) [M+NH_4^+], 355 (65) [M+2+H^+], 353 (100) [M+H^+], 351 (9) [M+H^+−2], *Anal* Calcd. for $C_{20}H_{16}O_6$: C 68.18 %, H 4.58 %, Found: C 68.12 %, H 4.32 %.

4-*O*-Acetyl-rubiginone D_2 (**3**)

MP 52°C; $[\alpha]_D^{22}$ +82° (*c* 0.12, MeOH); UV (MeOH) λ_{max} nm (lg ε) 262 (4.41), 378 (3.59); IR ν_{max} (KBr) cm^{-1} 3448 (br), 2926, 1738, 1711, 1671, 1589, 1296, 1270, 1227, 1034; CD (MeOH) λ_{extr} nm ([θ]) 234 (+1.73×10^4), 248 (−1.58×10^3), 271 (+3.43×10^4), 293 (−3.06×10^3), 323 (+8.76×10^3), 397 (−4.18×10^3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (d, *J*=7.2 Hz, 3H, 3-CH_3), 2.09 (s, 3H, $OCOCH_3$), 2.86 (qdd, *J*=7.2, 5.3, 2.5 Hz, 1H, 3-H), 3.67 (d, *J*=4.4 Hz, 1H, 2-OH), 4.03 (s, 3H, 8-OCH_3), 5.34 (dd, *J*=5.3, 4.4 Hz, 1H, 2-H), 6.02 (d, *J*=2.5 Hz, 1H, 4-H), 7.32 (dd, *J*=8.0, 1.7 Hz, 1H, 9-H), 7.69~7.79 (m, 3H, 5-H, 10-H, 11-H), 8.40 (d, *J*=8.0 Hz, 1H, 6-H); ^{13}C NMR (see Table 1); DCI-MS *m/z* (%) 429 (14) [M+NH_3+NH_4^+], 412 (16) [M+NH_4^+], 395 (7) [M+H^+], 369 (21), 335 (100) [M+H^+−C_2H_2O−H_2O].

4-*O*-Acetyl-2-*O*-[(*S*)-2′-phenylbutyryl]rubiginone D_2 (**4**)

A solution of 27 mg of **3**, 45 mg of (*S*)-2-phenylbutyric acid, 42 mg of dicyclohexylcarbodiimide and 25 mg of 4-(dimethylamino)pyridine in CH_2Cl_2 (10 ml) was stirred for 2 hours at room temperature. After the addition of CH_2Cl_2 (50 ml), the mixture was extracted two times with 0.05 M HCl (50 ml) and once with water (50 ml). The organic layer was evaporated to dryness and chromatographed on silica gel (CH_2Cl_2 - acetone 95 : 5) and Sephadex LH-20 (acetone) to yield 24 mg (65%) of **4**. MP 77°C; Rf 0.65 ($CHCl_3$ - MeOH, 9 : 1), 0.55 (acetone - cyclohexane, 2 : 1); $[\alpha]_D^{22}$ +22° (*c* 0.99, $CHCl_3$); IR λ_{max} (KBr) cm^{-1} 1741, 1673, 1590; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.92 (dd, *J*=7.5, 7.5 Hz, 3H, 4′-H_3), 0.98 (d, *J*=7.2 Hz, 3H, 3-CH_3), 1.89 (qdd, *J*=15.0, 7.5, 7.5 Hz, 1H, 3′-H_a), 2.12 (s, 3H, $OCOCH_3$), 2.22 (qdd, *J*=15.0, 7.5, 7.5 Hz, 1H, 3′-H_b), 2.74 (qdd, *J*=7.2, 4.5, 4.5 Hz, 1H, 3-H), 3.58 (dd, *J*=7.5, 7.5 Hz, 1H, 2′-H), 4.02 (s, 3H, 8-OCH_3), 5.95 (d, *J*=4.5 Hz, 1H, 2-H), 5.99 (d, *J*=4.5 Hz, 1H, 4-H), 7.13 (ddm, *J*=7.5, 7.5 Hz, 1H, 4″-H), 7.18 (dd, *J*=7.5, 7.5 Hz, 2H, 3″-H, 5″-H), 7.27 (dm, *J*=7.5 Hz, 2H, 2″-H, 6″-H), 7.29 (d, *J*=8.0 Hz, 1H, 9-H), 7.59 (d, *J*=8.0 Hz, 1H, 5-H), 7.70 (dd, *J*=8.0, 8.0 Hz, 1H, 10-H), 7.77 (d, *J*=8.0 Hz, 1H, 11-H), 8.31 (d, *J*=8.0 Hz, 1H, 6-H); EI-MS *m/z* (%) 540.1784 (0.3) [M^+, calcd. for $C_{32}H_{28}O_8$ and found], 394 (13), 119 (22), 91 (39), 60 (58), 43 (100).

4-*O*-Acetyl-2-*O*-[(*R*)-2′-phenylbutyryl]rubiginone D_2 (**5**)

In an analogous procedure as described for the synthesis of **4** 28 mg of **3** was esterified with (*R*)-2-phenylbutyric acid and purified to give 24 mg (62%) of **5**. MP 77°C; Rf 0.65 ($CHCl_3$ - MeOH, 9 : 1), 0.55 (acetone - cyclohexane, 2 : 1); $[\alpha]_D^{22}$ +13° (*c* 0.99, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 1742, 1673, 1590; ^1H NMR (500 MHz, $CDCl_3$) δ 0.80 (d, *J*= 7.0 Hz, 3H, 3-CH_3), 0.94 (dd, *J*=7.5, 7.5 Hz, 3H, 4′-H_3), 1.87 (qdd, *J*=13.5, 7.5, 7.5 Hz, 1H, 3′-H_a), 2.10 (s, 3H, $OCOCH_3$), 2.22 (qdd, *J*=13.5, 7.5, 7.5 Hz, 1H, 3′-H_b), 2.66 (qdd, *J*=7.0, 4.5, 3.5 Hz, 1H, 3-H), 3.63 (dd, *J*=7.5, 7.5 Hz, 1H, 2′-H), 4.02 (s, 3H, 8-OCH_3), 5.95 (d, *J*=3.5 Hz, 1H, 4-H), 6.11 (d, *J*=4.5 Hz, 1H, 2-H), 7.22 (dddd, *J*=7.5, 7.5, 1.5, 1.5 Hz, 1H, 4″-H), 7.27 (dd, *J*=7.5, 7.5 Hz, 2H, 3″-H, 5″-H), 7.29 (dd, *J*=8.0, 1.0 Hz, 1H, 9-H), 7.32 (dm, *J*= 7.5 Hz, 2H, 2″-H, 6″-H), 7.66 (d, *J*=8.0 Hz, 1H, 5-H), 7.70 (dd, *J*=8.0, 8.0 Hz, 1H, 10-H), 7.77 (dd, *J*=8.0, 1.0 Hz, 1H, 11-H), 8.35 (d, *J*=8.0 Hz, 1H, 6-H); EI-MS *m/z* (%) 540.1784 (2) [M^+, calcd. for $C_{32}H_{28}O_8$ and found], 394 (17), 334 (29) 119 (28), 91 (56), 43 (100).

Rubiginon H (**6**)

MP 151°C; $[\alpha]_D^{22}$ −223° (*c* 0.19, MeOH); UV (MeOH) λ_{max} nm (lg ε) 260 (3.86), 298 (3.62), 335 (3.73); IR ν_{max} (KBr) cm^{-1} 3448 (br), 1770, 1748, 1646, 1614, 1493, 1276, 1228, 1043; CD (MeOH) λ_{extr} nm ([θ]) 225 ($+7.52\times10^3$), 233 (-4.37×10^3), 237 ($+6.26\times10^3$), 260 (-2.32×10^4), 301 ($+1.57\times10^3$), 340 (-7.13×10^3); ^1H NMR (300 MHz, $CDCl_3$) δ 0.93 (d, *J*=7.0 Hz, 3H, 3-CH_3), 2.05 (s, 3H, 4-$OCOCH_3$), 2.80 (qdd, *J*=7.0, 5.0, 2.5 Hz, 1H, 3-H), 3.48 (d, *J*=1.0 Hz, 1H, 2-OH), 3.75 (s, 3H, 8-OCH_3), 4.95 (dd, *J*=5.0, 1.0 Hz, 1H, 2-H), 5.94 (d, *J*=2.5 Hz, 1H, 4-H), 6.77 (s, 1H, 7-H), 6.90 (d, *J*=8.0 Hz, 1H, 5-H), 7.07~7.13 (m, 1H, 9-H), 7.12 (d, *J*=8.0 Hz, 1H, 6-H), 7.50~7.56 (m, 2H, 10-H, 11-H), 11.95 (s, 1H, 12a-OH); ^{13}C NMR (see Table 1); EI-MS *m/z* (%) 412.1158 (13) [M^+, calcd. for $C_{22}H_{20}O_8$ and found], 370 (8) [$M^+-C_2H_2O$], 352 (100) [$M^+-C_2H_2O-H_2O$], 334 (40), 323 (27), 163 (38), 43 (33) [$C_2H_3O^+$].

Rubiginon I (**7**)

MP 132°C; $[\alpha]_D^{22}$ +197° (*c* 0.72, MeOH); UV (MeOH) λ_{max} nm (lg ε) 257 nm (3.85), 295 (3.77); IR ν_{max} (KBr) cm^{-1} 3440 (br), 1735, 1700, 1692, 1261, 1229, 1046; CD (MeOH) λ_{extr} nm ([θ]) 230 (-1.67×10^4), 263 ($+3.72\times10^4$), 316 ($+1.02\times10^4$), 359 ($+2.46\times10^3$); ^1H NMR (300 MHz, $CDCl_3$): δ=0.84 (d, *J*=7.2 Hz, 3H, 3-CH_3), 2.13 (s, 3H, 4-$OCOCH_3$), 2.45 (s br, 1H, 7-OH), 2.71 (qdd, *J*= 7.2, 5.4, 2.0 Hz, 1H, 3-H), 3.60 (d, *J*=3.0 Hz, 1H, 2-OH), 3.93 (s, 3H, 8-OCH_3), 5.07 (dd, *J*=5.4, 3.0 Hz, 1H, 2-H), 5.58 (d, *J*=2.0 Hz, 1H, 4-H), 5.92 (s, 1H, 7-H), 6.57 (d, *J*= 9.6 Hz, 1H, 5-H), 7.05 (d, *J*=9.6 Hz, 1H, 6-H), 7.13 (dd, *J*= 8.3, 1.0 Hz, 1H, 9-H), 7.38 (dd, *J*=7.5, 1.0 Hz, 1H, 11-H), 7.46 (dd, *J*="7.8, 7.8" Hz, 1H, 10-H); ^{13}C NMR (see Table 1); EI-MS *m/z* (%) 412.1158 (3) [M^+, calcd. for $C_{22}H_{20}O_8$ and found], 352 (73) [$M^+-C_2H_2O-H_2O$], 334 (100), 307 (49), 305 (44), 163 (36), 43 (34) [$C_2H_3O^+$].

Antitumor Activity

The antitumor activity of the test compounds was determined in four human cancer cell lines, according to the NCI guidelines[15]. The cell lines used were HMO2 (stomach adenocarcinoma), Kato III (colon carcinoma), HEP G2 (hepatocellular carcinoma) and MCF 7 (breast adenocarcinoma). Cells were grown in 96-well microtitre plates of RPMI tissue culture medium supplemented with 10% fetal calf serum at 37°C in a humified atmosphere of 5% CO_2 in air. After 24 hours of incubation the test compounds (0.1~50 μmol/liter) were added to the cells. Stock solutions of the test compounds were prepared in MeOH. After a 48 hours incubation in the presence of the test drugs the cells were fixed by addition of trichloroacetic acid and cell protein was assayed with sulforhodamine B[16]. For each compound tested the GI_{50} (drug concentration causing 50% growth inhibition) and TGI values (drug concentration causing 100% growth inhibition) were determined.

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