METABOLITES OF MICROORGANISMS. 248†
SYNTHETIC ANALOGS OF SAPHENAMYCIN

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A synthesis of racemic saphenic acid is described. From this acid 9 ester derivatives of saphenamycin were prepared. Those with aromatic acid components showed high activity against many Gram-positive and some Gram-negative bacteria. Of the esters with aliphatic acid moieties only the acetate and, to a lesser extent, the butyrate showed considerable antibacterial activities, whereas esters with higher fatty acids showed strongly reduced, if any, activities against some test organisms. Similar results were obtained with ID_{50} values against the eucaryotic tumor cell line CCRF/CEM. The salicylate, which is structurally similar to saphenamycin, was most active.

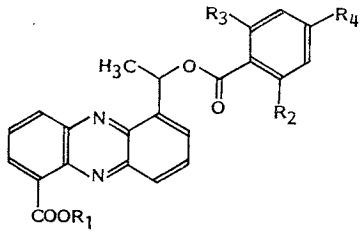
We recently isolated saphenamycin (**1**), together with some related phenazines[1], and observed that a mixture of saphenyl esters of higher aliphatic fatty acids was nearly inactive compared with the high antibacterial activity of saphenamycin itself[2]. In order to assess some structure-activity relationships we synthesized a series of esters analogous to saphenamycin with aliphatic and aromatic side chains.

Despite the modest yields reported for the WOHL-AUE reaction[3~5] we chose this method for the synthesis of saphenic acid (**2**)[1,6,7] because it allowed the preparation of suitably substituted phenazines in a single reaction from easily available starting materials. Heating of anthranilic acid (**3**) with the ethylene acetal (**4**), prepared from commercial *o*-nitroacetophenone, gave phenazine **5** in *ca.* 5% yield. One of the reasons for this low yield is a known side reaction[8], the formation of the azobenzene derivative (**6**). This compound was not isolated but identified *via* 2-carboxy-2′-hydroxyethylazobenzene (**7**) which was characterized as the acetate **8**.

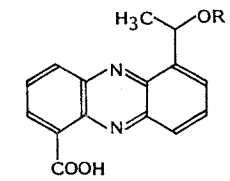
The phenazine **5** was deprotected with acetone and *p*-toluenesulfonic acid. The product, 6-acetylphenazine-1-carboxylic acid (**9**), was identical in all properties with the natural product[1]. Reduction of **9** with $NaBH_4$ furnished racemic saphenic acid (**2**) which was identical with the natural product[1].

From synthetic saphenic acid a series of esters were prepared by conventional methods. Acetyla-

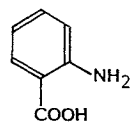
† Preceding paper see ref 1.



1	$R_1=H$	$R_2=OH$	$R_3=CH_3$	$R_4=H$
18	$R_1=H$	$R_2=H$	$R_3=H$	$R_4=Br$
19	$R_1=H$	$R_2=H$	$R_3=CH_3$	$R_4=H$
20	$R_1=H$	$R_2=OH$	$R_3=H$	$R_4=H$
21	$R_1=H$	$R_2=OCH_3$	$R_3=CH_3$	$R_4=H$
22	$R_1=CH_3$	$R_2=OCH_3$	$R_3=CH_3$	$R_4=H$



2	$R=H$
10	$R=COCH_3$
11	$R=COCH_2CH_2CH_3$
12	$R=CO(CH_2)_6CH_3$
13	$R=CO(CH_2)_{14}CH_3$
14	$R=CO(CH_2)_7CH=CH(CH_2)_7CH_3$



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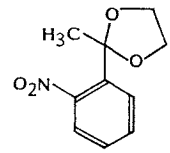

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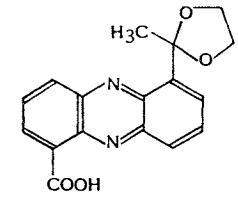

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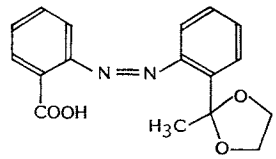

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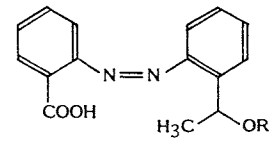
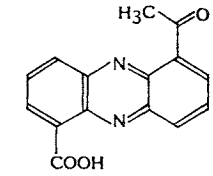


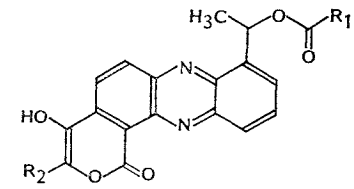
7 R = H

8 R = $COCH_3$



9

tion was carried out with acetic anhydride and pyridine, yielding the crystalline acetate **10** and, surprisingly, the ketoacid **9**. This product was not formed by an oxidation process, but by substitution of the hydroxyethyl side chain by an acetyl residue. When the reaction was carried out with hexadeuteroacetic anhydride, the corresponding compound was deuterated to a high degree, although a part of the deuterium was lost during working up.



15	$R_1=C_7H_{15}$	$R_2=C_6H_{13}$
16	$R_1=C_{15}H_{31}$	$R_2=C_{14}H_{29}$
17	$R_1=C_{17}H_{33}$	$R_2=C_{16}H_{31}$

Higher fatty acid esters (butyrate **11**, caprylate **12**, palmitate **13**, oleate **14**) were prepared with the corresponding acid chlorides in the presence of 4-dimethylaminopyridine. In the latter three cases,

Table 1. ^{1}H NMR spectra of synthetic saphenyl esters (300 MHz, $CDCl_3$) (signals of the side chain protons, J in Hz).

Acetate (**10**)	Butyrate (**11**)	Caprylate (**12**)	Palmitate (**13**)	Oleate (**14**)
7.21 (1H, q, J=6.5)	7.22 (1H, q, J=6.5)	7.22 (1H, q, J=6.5)	7.22 (1H, q, J=6.5)	7.22 (1H, q, J=6.5)
2.19 (3H, s)	2.43 (2H, m)	2.44 (1H, t, J=7.6)	2.44 (1H, t, J=7.7)	5.34 (2H, m)
1.76 (3H, d, J=6.5)	1.76 (3H, d, J=6.5)	2.43 (1H, t, J=7.6)	2.43 (1H, t, J=7.3)	2.44 (1H, t, J=7.6)
	1.75 (2H, m)	1.76 (3H, d, J=6.5)	1.76 (3H, d, J=6.5)	2.43 (1H, t, J=7.3)
	0.99 (3H, t, J=7.4)	1.68 (2H, m)	1.68 (2H, m)	2.00 (4H, m)
		1.4~1.2 (8H, br s)	1.4~1.2 (24H, br s)	1.76 (3H, d, J=6.5)
		0.87 (3H, t, J=6.8)	0.87 (3H, t, J=6.7)	1.68 (2H, m)
				1.4~1.2 (20H, br s)
				0.87 (3H, t, J=6.9)

p-Bromobenzoate (**18**)	Toluylate (**19**)	Salicylate (**20**)	2-Methoxy-6-methyl-benzoate (**21**)
7.44 (1H, q, J=6.5)	8.1 (4H, m, overlapping)	10.64 (1H, s, exchangeable)	7.49 (1H, q, J=6.6)
8.05 (2H, m, overlapping with signals of the phenazine protons)	7.45 (2H, m, overlapping)	8.05 (4H, m, overlapping)	7.27 (1H, t, J=8.2)
		7.49 (1H, q, J=6.6)	6.82 (1H, d, J=8.2)
			6.80 (1H, d, J=8.2)
7.63 (2H, m)	7.30 (2H, m)	6.97 (2H, m)	3.81 (3H, s)
1.96 (3H, d, J=6.5)	2.62 (3H, s)	1.93 (3H, d, J=6.6)	2.32 (3H, s)
	1.90 (3H, d, J=6.6)		1.88 (3H, d, J=6.6)

All compounds show the following signals of protons of the phenazinecarboxylic acid nucleus: 15.5 (1H, s or br s, exchangeable), 8.98 (1H, dd, J=7.1 and 1.4 Hz), 8.56 (1H, dd, J=8.7 and 1.4 Hz), 8.20 (1H, m or dd, J=8.4 and 1.4 Hz), 8.03 (1H, dd, J=8.7 and 7.1 Hz), 8.00 (2H, m); deviations within 0.02 ppm in δ, 0.1 Hz in J. In the spectra of **18** to **20** some of these signals are obscured by overlapping with side chain signals.

in addition to the saphenyl esters, purple side products were obtained. The purple compound **15** produced with caprylic acid chloride was studied in some detail. The MS (M^+ 518 for $C_{31}H_{38}N_2O_5$) indicated that saphenic acid had reacted with 2 mol of the acid chloride. One of them had obviously formed an ester with the alcoholic group of saphenic acid (**2**). An acidic proton gave a ^1H NMR signal at 13.65 ppm, considerably upfield from that of phenazine-1-carboxylic acids (15.5 ppm, Table 1). The lack of a signal of a proton at C-2 of the phenazine nucleus (8.9 ppm) and the presence of an AB signal group (7.54 and 7.35 ppm, J=10 Hz) for the protons at C-3 and C-4 of the phenazine nucleus indicated that position 2 of saphenic acid was substituted. The second alkyl side chain is shorter by one CH_2 group. The spectra are in best agreement with structure **15**. To the purple side products, formed with palmitic and oleic acid chloride, were assigned structures **16** and **17** respectively, in view of their spectroscopic properties.

The aromatic saphenyl esters **18** and **19** were difficult to purify and needed several chromatographic procedures, although no purple side products were formed in these cases.

Activated acid derivatives of salicylic acid with a protected phenolic OH group (OCH_2OCH_3 or $OSi(CH_3)_3$) did not react with saphenic acid under usual conditions. Finally, a mixture prepared by the reaction of salicylic acid and $SOCl_2$ which consisted, according to an MS analysis, of salicylic acid chloride and condensation products (esters) with two and three salicylic acid residues gave, upon reaction with racemic saphenic acid, a product, from which yellow crystals were isolated, whose elemental composition and ^1H NMR spectrum were in full agreement with structure **20**.

6-Methylsalicylic acid and its hydroxyl derivatives did not react with $SOCl_2$ under usual conditions. The synthesis of saphenamycin is therefore still an unsolved problem. 2-Methoxy-6-methylbenzoic acid, on the other hand, gave smoothly an acid chloride, which reacted with racemic saphenic acid to give racemic saphenamycin methyl ether (**21**). Its methyl ester (**22**), prepared with CH_2N_2, was identical with a sample prepared from natural saphenamycin[1] according to TLC and its ^1H NMR spectrum.

Biological Activities

The determination of the antimicrobial and antitumor activity will be fully described in a separate paper[8]. Some MIC values are listed in Table 2. As with the antimicrobial activity, the esters with aromatic acids showed a higher antitumor activity than the esters with higher aliphatic acids. The

Table 2. MIC values (μg/ml) of saphenyl esters.

Microorganism	**1** (natural)	**20**	**19**	**18**	**10**	**11**	**14**
Bacillus brevis[8]	0.01	0.02	—	—	0.009	0.02	0.9
B. cereus	0.07	0.003	0.1	0.01	0.1	<0.1	—
B. subtilis[a]	0.001	0.079	0.08	<0.1	—	—	—
Corynebacterium glutamicum	0.2	<0.001		—	—	—	—
Streptomyces glaucescens	—	0.22	0.16	—	0.15	0.51	—
S. viridochromogenes	0.35	0.13	—	—	—	—	—
Proteus mirabilis	<0.01	<0.001	0.002	<0.001	0.003	0.001	—
P. vulgaris	0.005	<0.001	0.5	0.5	0.5	0.5	—
Xanthomonas campestris	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001

[a] Defined media; all others were complex media.

—: No activity.

The caprylate (**12**) and the palmitate (**13**) were inactive except for *Xanthomonas campestris*.

ID_{50} values against the tumor cell line CCRF/CEM are 18 μg/ml for the *p*-bromobenzoate (**18**), 4 μg/ml for the salicylate (**20**) and 7.4 μg/ml for the acetate (**10**), but are much higher for the esters **11**, **12**, **13** and **14**[8]. The aromatic ring system of the ester seems to be important for the biological activity of the phenazines. Therefore, further modifications are in progress.

Experimental

(A) General Procedure

Silica gel impregnated with oxalic acid for flash chromatography was prepared as follows: Silica gel 60 (Merck, 500 g) was suspended in a solution of oxalic acid (9 g) in ether (1 liter). After 1 hour the ether was removed at low pressure and the silica gel dried in the air (2 hours) and then heated to 120°C for 30 minutes. To remove oxalic acid from the eluates these were washed three times with water, dried with $MgSO_4$ and evaporated under reduced pressure.

For TLC silica gel plates Merck F_{254} were used. If indicated they were impregnated with oxalic acid in ether and dried in the same way as above.

(B) Synthesis of Saphenic Acid

2-Methyl-2-(2-nitrophenyl)-1,3-dioxole (4)

From 100 g of 2-nitroacetophenone, 47.2 g of ethylene glycol and 500 mg of *p*-toluenesulfonic acid in 200 ml abs benzene the ethylene ketal **4** was prepared by 16 hours refluxing and continuous removal of water. The mixture was diluted with 300 ml of $CHCl_3$, washed twice with water and dried with $MgSO_4$. The solvents were removed under reduced pressure. After cooling to room temperature the residue solidified and was recrystallized from acetone - hexane, 120.3 g (95%), colorless crystals: MP 66°C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.86 (3H, s), 3.66 (2H, m), 4.02 (2H, m), 7.41 (2H, m), 7.50 (1H, m), 7.66 (1H, dt, J_d=7.9 Hz, J_t=0.9 Hz).

Anal Calcd for $C_{10}H_{11}NO_4$: C 57.41, H 5.30, N 6.70.
Found: C 57.22, H 5.31, N 6.64.

6-(2-Methyl-1,3-dioxol-2-yl)-1-phenazinecarboxylic Acid (5)

A mixture of 12 g of finely ground anthranilic acid (**3**), 12 g of pulverized KOH, 20 g of ethylene ketal **4**, and 50 g of quartz sand in a 500-ml round bottomed flask was rotated in an oil bath in oblique position and heated to 80°C. After 30 minutes the temperature was raised slowly to 200°C within 3.5 hours. The flask was then rotated for an additional 10 minutes at this temperature. The dark brown to black mixture was cooled to *ca.* 100°C and then 200 ml of water was slowly added. After cooling to room temperature the mixture was filtered through glass wool and the filtrate extracted with 250 ml of $CHCl_3$. The organic extract was discarded and the aqueous phase acidified with 170 ml of 6 N HCl (pH 1). The product was extracted 8 times with $CHCl_3$ (150 ml each), the extract washed twice with 2 N HCl and 4 times with water. After drying (Na_2SO_4) and evaporation under reduced pressure 6.5 g of a black oil was obtained. By chromatography on 100 g Florisil (60~100 mesh, elution with $CHCl_3$ - acetone, 1 : 1) 2.09 g of a fraction was obtained which solidified to red crystals after evaporation. Further impurities were removed by flash chromatography on silica gel impregnated with oxalic acid ($CHCl_3$). The main fractions gave by recrystallization from acetone - hexane, 1.42 g (5%) of **5** as light yellow needles: MP 172~173°C; ^1H NMR (300 MHz, $CDCl_3$) δ 2.22 (3H, s), 3.96 (2H, m), 4.20 (2H, m), 7.96 (1H, dd, J=7.1 and 8.6 Hz), 8.04 (1H, dd, J=7.2 and 8.7 Hz), 8.21 (1H, dd, J=7.2 and 1.4 Hz), 8.24 (1H, dd, J=8.6 and 1.3 Hz), 8.65 (1H, dd, J=8.7 and 1.4 Hz), 8.97 (1H, dd, J=7.1 and 1.3 Hz), 15.54 (1H, br s, exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.1 (q), 64.8 (2C, t), 108.6 (s), 124.3 (s), 128.0 (d), 128.4 (d), 129.8 (d), 132.1 (d), 135.6 (d), 137.3 (d), 139.0 (s), 140.1 (s), 140.5 (s), 142.0 (s), 142.1 (s), 165.6 (s); electron impact mass spectra (EI-MS) *m/z* (relative intensity) 310 (16, M^+), 296 (20), 295 (100), 268 (24), 267 (100), 266 (37), 251 (21), 223 (10), 207 (11), 206 (9), 205 (26), 194 (17), 180 (7), 179 (33), 152 (8), 103 (8), 87 (69), 76 (9), 75 (12), 63 (7), 51 (6), 43 (45).

Anal Calcd for $C_{17}H_{14}N_2O_4$: C 65.80, H 4.55, N 9.03.
Found: C 65.84, H 4.31, N 9.09.

Racemic 2-[[2-(1-Hydroxyethyl)phenyl]azo]benzoic Acid (7)

Orange to red-brown colored side fractions from the chromatographic purification of **5** were refluxed for 4 hours in 100 ml of abs acetone and 1.3 g of *p*-toluenesulfonic acid. The solution was then concentrated under reduced pressure, the residue dissolved in 200 ml of $CHCl_3$ and washed 3 times with water. Evaporation of the dried solution gave a brownish red oil which was dissolved in 200 ml of MeOH - THF (1 : 1). After the addition of 5 g of $NaBH_4$ the mixture was stirred for 5 hours at room temperature, the solution was acidified with 2 N HCl to pH *ca.* 6, concentrated under reduced pressure and diluted with H_2O to *ca.* 1 liter. The solution was extracted with $CHCl_3$ and the dried and evaporated extract chromatographed on Sephadex LH-20 with $CHCl_3$ - MeOH (1 : 1). Two fractions were eluted, 752 mg of **7** (red prisms after recrystallization from acetone - hexane) and 150 mg of saphenic acid (**2**) as yellow needles (see below). The red crystals showed mp 160~161°C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.60 (3H, d, J=6.5 Hz), 5.85 (1H, q, J=6.5 Hz), 6.3~6.5 (2H, br), 7.40~7.48 (1H, m), 7.50 (1H, dd, J=8.1 and 1.3 Hz), 7.59~7.68 (1H, m), 7.68~7.76 (2H, m), 7.78 (1H, dd, J=7.78 and 1.1 Hz), 7.85~7.95 (1H, m), 8.40~8.50 (1H, m); UV λ_{max}^{EtOH} nm (ε) 206 (20,690), 236 (sh, 11,300), 323 (12,940); EI-MS *m/z* (relative intensity) 270 (4, M^+), 253 (12), 252 (36), 237 (46), 235 (49), 210 (14), 208 (13), 207 (23), 181 (7), 178 (6), 152 (7), 137 (7), 121 (28), 120 (15), 119 (15), 105 (19), 104 (16), 103 (44), 93 (22), 92 (15), 91 (29), 77 (100), 76 (22), 65 (61), 63 (16), 51 (40), 50 (21), 45 (10), 43 (29), 39 (37), 27 (10), 18 (7).

Anal Calcd for $C_{15}H_{14}N_2O_3$: C 66.65, H 5.22, N 10.36.
Found: C 66.34, H 5.30, N 10.36.

The acetylation product **8** (from 100 mg **7** in 4 ml Ac_2O - pyridine (1 : 1), 16 hours, room temperature) gave after chromatography on Sephadex LH-20, red prisms: ^1H NMR (300 MHz, $CDCl_3$) δ 1.63 (3H, d, J=6.6 Hz), 2.13 (3H, s), 6.95 (1H, q, J=6.6 Hz), 7.42~7.55 (2H, 8m), 7.60~7.80 (4H, m), 8.00~8.07 (1H, m), 8.43~8.50 (1H, m), 13.00 (1H, br s, exchangeable).

6-Acetyl-1-phenazinecarboxylic Acid (9)

A solution of 2.6 g **5** and 100 mg of *p*-toluenesulfonic acid in 250 ml of abs acetone was refluxed for 3 hours. The solution was then concentrated under reduced pressure and the residue dissolved in $CHCl_3$ (600 ml). The solution was washed twice with H_2O and once with satd NaCl, dried with Na_2SO_4 and evaporated. The solid residue was recrystallized from acetone - hexane and gave fine yellow crystals, 1.9 g, 75%, mp 218~219°C, no depression on admixture with natural **9**[1]. ^1H NMR, ^{13}C NMR, IR and MS are the same as those of the natural compound.

Anal Calcd for $C_{15}H_{10}N_2O_3$: C 67.67, H 3.79, N 10.52.
Found: C 67.28, H 3.86, N 10.35.

Racemic 6-(1-Hydroxyethyl)-1-phenazinecarboxylic Acid (Racemic Saphenic Acid) (2)

A solution of 1.2 g of $NaBH_4$ in 25 ml of THF - MeOH (1 : 1) was slowly dropped into 1 g of **9** in 500 ml of THF - MeOH. The mixture was stirred for 30 minutes at room temperature and then 30 minutes at 45°C. The cooled solution (20°C) was acidified (pH 6) with 2 N HCl, concentrated under reduced pressure, diluted with H_2O (1 liter) and extracted 4 times with $CHCl_3$. The extracts were dried (Na_2SO_4), evaporated under reduced pressure, and the residue recrystallized from acetone - hexane. Yellow needles (800 mg, 79%) were obtained, mp 205~206°C (dec). The spectra (IR, ^1H NMR and MS) and Rf values (TLC, $CHCl_3$ - acetone (4 : 1), Rf 0.41; hexane - acetone (1 : 1), Rf 0.27) were identical with those of natural saphenic acid[1].

Anal Calcd for $C_{15}H_{12}N_2O_3$: C 67.16, H 4.51, N 10.44.
Found: C 67.05, H 4.47, N 10.43.

The methyl ester of synthetic saphenic acid was prepared from **2** (312 mg) in 15 ml of $CHCl_3$ by the addition of 10 ml of ethereal CH_2N_2 (3%) and stirring for 1 hour at room temperature. After evaporation the crude product was chromatographed on 35 g of silica gel (hexane - acetone, 1 : 1) and the product recrystallized from acetone - hexane, 251 mg (76%), light yellow prisms, mp 162~163°C. The optically active ester prepared from natural saphenic acid had mp 151~152°C[1]. IR, ^1H NMR, MS and Rf were identical with those of the ester prepared from natural saphenic acid[1].

(C) Syntheses of Saphenyl Esters

Racemic 6-[1-(Acetyloxy)ethyl]-1-phenazinecarboxylic Acid (**10**)

Racemic saphenic acid (**2**, 112 mg) in 4 ml Ac_2O - pyridine (1 : 1) was stirred for 16 hours at room temperature and then heated to reflux for 3 hours. The mixture was dissolved in 25 ml $CHCl_3$, washed with satd $CuSO_4$, H_2O and satd NaCl and then dried with Na_2SO_4. After evaporation under reduced pressure the brown oil (153 mg) was separated by chromatography (35 g silica gel, hexane - acetone (1 : 1), flash method) into two products. The first one was recrystallized from acetone - hexane; 82 mg of greenish yellow platelets of **10**: MP 204°C (dec); ^{1}H NMR Table 1; ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.7 (q), 22.2 (q), 67.7 (d), 124.5 (s), 126.7 (d), 127.0 (d), 129.9 (d), 132.7 (d), 135.3 (d), 137.3 (d), 139.4 (s), 139.6 (s), 141.0 (s), 142.2 (s), 165.6 (s), 169.8 (s); EI-MS *m/z* (relative intensity) 310 (8, M^+), 268 (40), 267 (100), 266 (74), 251 (30), 250 (28), 224 (7), 223 (9), 222 (18), 207 (30), 206 (30), 205 (74), 181 (13), 180 (16), 179 (26), 153 (9), 152 (9), 103 (17), 102 (11), 77 (15), 76 (11), 75 (13), 63 (9), 50 (9), 43 (66).

The second product isolated by chromatography (38 mg) proved to be identical with 6-acetylphenazine-1-carboxylic acid (**9**) by IR, ^{1}H NMR, and TLC. By the application of hexadeutero acetic anhydride in the acetylation reaction both products were obtained in deuterated form (^{1}H NMR and EI-MS). Deuterated **9**: ^{1}H NMR; instead of δ 3.07 (3H, s) *ca.* 7 peaks, total intensity *ca.* 1H; all other signals as in the natural compound[1]; EI-MS *m/z* (relative intensity) 269 (12), 268 (15).

Racemic 6-[1-(1-Oxobutoxy)ethyl]-1-phenazinecarboxylic Acid (**11**)

To saphenic acid (209 mg) and 4-dimethylaminopyridine (150 mg) in abs $CHCl_3$ (10 ml) a solution of butyryl chloride (500 mg) was added and the mixture stirred for 16 hours at room temperature. After dilution with 50 ml of $CHCl_3$ the solution was washed 3 times with H_2O and once with satd NaCl and then dried with Na_2SO_4. After evaporation, the product was chromatographed on 80 g of silica gel impregnated with oxalic acid and then on Sephadex LH-20 (acetone as eluent). Recrystallization from acetone - hexane gave 117 mg of **11** (44%) as yellow needles: MP 150~151°C; ^{1}H NMR Table 1; ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7 (q), 18.6 (t), 22.3 (q), 36.5 (t), 67.5 (d), 124.9 (s), 126.9 (d), 127.2 (d), 130.1 (d), 132.9 (d), 135.4 (d), 137.5 (d), 139.7 (s), 139.9 (s), 141.3 (s), 142.2 (s), 142.5 (s), 165.8 (s), 172.6 (s); EI-MS *m/z* (relative intensity) 338 (6.3, M^+).

Anal Calcd for $C_{19}H_{18}N_2O_4$: C 67.45, H 5.36, N 8.28.
Found: C 67.17, H 5.31, N 8.08.

Racemic 6-[1-(1-Oxooctyloxy)ethyl]-1-phenazinecarboxylic Acid (**12**)

In the same manner 200 mg of racemic saphenic acid was reacted with 500 mg of caprylic acid chloride and the crude product separated by chromatography on oxalic acid impregnated silica gel into a purple side product (178 mg) and the caprylate **12** (145 mg). The fraction containing **12** gave, after an additional chromatography on Sephadex LH-20 and recrystallization from acetone - hexane 98 mg of yellow crystals: MP 149~150°C; ^{1}H NMR Table 1; EI-MS *m/z* (relative intensity) 394 (0.3, M^+).

Anal Calcd for $C_{23}H_{26}N_2O_4$: C 70.03, H 6.64, N 7.10.
Found: C 70.13, H 6.64, N 7.09.

The purple side product (**15**) was recrystallized from acetone - hexane: MP 149~150°C; UV λ_{max}^{EtOH} nm (ε) 221 (28,080), 324 (17,510), 535 (21,670), 568 (23,114), 608 (16,852); ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.65 (1H, br s, exchangeable), 7.65 (1H, t, J=8.0 Hz), 7.55 (1H, br d, J=8.0 Hz), 7.54 (1H, d, J=10.0 Hz), 7.40 (1H, dd, J=8.0 and 1.4 Hz), 7.35 (1H, d, J=10.0 Hz), 6.91 (1H, q, J=6.5 Hz), 2.67 (2H, t, J=7.6 Hz), 2.394 (1H, t, J=7.7 Hz), 2.391 (1H, t, J=7.0 Hz), 1.62 (3H, d, J=6.5 Hz), 1.72~1.60 (2H, m), 1.60~1.50 (2H, m), 1.50~1.20 (14H, br s), 0.89 (3H, t, J=6.7 Hz), 0.87 (3H, t, J=6.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.0 (s), 165.2 (s), 162.4 (s), 148.0 (s), 142.4 (s), 141.0 (s), 137.9 (s), 135.1 (s), 132.6 (d), 132.0 (d), 131.2 (d), 128.0 (s), 123.0 (d), 116.3 (d), 115.9 (s), 89.9 (s), 67.2 (d), 34.6 (t), 31.7 (2C, t), 30.0 (t), 39.2 (t), 29.1, 28.9 (t), 26.3 (t), 25.0 (t), 22.6 (t), 22.4 (t), 22.2 (q), 14.1 (2C, q); EI-MS *m/z* (relative intensity) 519 (23, M+1), 518 (65, M^+).

Racemic 6-[1-Oxohexadecyloxy)ethyl]-1-phenazinecarboxylic Acid (**13**)

From 200 mg of saphenic acid and 400 mg of palmitic acid chloride a mixture of a purple and a

yellow product was obtained and separated on Sephadex LH-20 (acetone) into 60 mg of the purple and 293 mg of the yellow compound. The latter was further purified by flash chromatography on 80 g of silica gel (hexane - acetone, 2 : 1). After an additional chromatography on Sephadex LH-20 ($CHCl_3$ - MeOH, 1 : 1) and recrystallization from acetone - hexane 135 mg (36%) of yellow platelets were obtained: MP 96~98°C; ^1H NMR Table 1; EI-MS *m/z* (relative intensity) 507 (0.4, M+1).

Anal Calcd for $C_{31}H_{42}N_2O_4$: C 73.49, H 8.35, N 5.53.
Found: C 73.22, H 8.47, N 5.63.

The purple fraction was again chromatographed on silica gel (hexane - acetone (3 : 1), flash) and then on Sephadex LH-20 ($CHCl_3$ - MeOH, 1 : 1). The dark purple powder (**16**) could not be crystallized: ^1H NMR (300 MHz, $CDCl_3$) δ 13.66 (1H, br s, exchangeable), 7.61 (1H, t, J=8 Hz), 7.55 (1H, dd, J=8.0 and 1.0 Hz), 7.54 (1H, d, J=10.0 Hz), 7.39 (1H, dd, J=8.0 and 1.0 Hz), 7.36 (1H, d, J=10.0 Hz), 6.91 (1H, q, J=6.6 Hz), 2.68 (2H, br t, J=7.5 Hz), 2.391 (1H, t, J=7.5 Hz), 2.388 (1H, t, J=7.3 Hz), 1.80~1.50 (4H, m), 1.62 (3H, d, J=6.6 Hz), 1.35~1.20 (48H, br), 0.95~0.80 (6H, m).

[Racemic (*Z*)]-6-[1-(1-Oxo-9-octadecenyloxy)ethyl]-1-phenazinecarboxylic Acid (**14**)

The reaction of racemic saphenic acid (103 mg) and oleic acid chloride gave again a purple (157 mg crude product) and a yellow product (82 mg,) which were separated by flash chromatography (80 g silica gel, hexane - acetone, 3 : 2). The yellow compound was further purified on a column of Sephadex LH-20 ($CHCl_3$ - MeOH, 1 : 1) and then on 35 g of silica gel (hexane - acetone (2 : 1), flash). The final product was a yellow powder: MP 71~72°C; ^1H NMR Table 1; EI-MS *m/z* (relative intensity) 532 (0.7, M^+).

The purple component (**17**) was again chromatographed on Sephadex LH-20 ($CHCl_3$ - MeOH, 1 : 1) and then on a silica gel column (hexane - acetone (3 : 1), flash) and finally purified by preparative TLC. The dark purple powder (27 mg) was homogeneous (TLC), but could not be crystallized: ^1H NMR (300 MHz, $CDCl_3$) δ 13.66 (1H, br s, exchangeable), 7.65 (1H, t, J=7.9 Hz), 7.55 (1H, br d, J=7.9 Hz), 7.54 (1H, d, J=10.0 Hz), 7.40 (1H, dd, J=7.9 and 1.3 Hz), 7.36 (1H, d, J=10.0 Hz), 6.91 (1H, q, J=6.5 Hz), 5.45 (4H, m), 2.67 (2H, br t, J=7.6 Hz), 2.40 (1H, t, J=7.7 Hz), 2.395 (1H, t, J=7.0 Hz), 2.10~1.90 (8H, m), 1.80~1.50 (4H, m), 1.62 (3H, d, J=6.5 Hz), 1.45~1.18 (36H, br), 0.90~0.80 (6H, m).

Racemic 6-[1-(4-Bromobenzoyloxy)ethyl]-1-phenazinecarboxylic Acid (**18**)

From 200 mg racemic saphenic acid, 175 mg *p*-bromobenzoyl chloride and 300 mg *p*-dimethylaminopyridine in 10 ml CH_2Cl_2 613 mg of a yellow oil was obtained after usual working up. The crude product was first chromatographed on silica gel (hexane - acetone, 1 : 1) and then twice on Sephadex LH-20 ($CHCl_3$ - MeOH, 1 : 1). After removal of impure fractions 32 mg of a homogeneous product (**18**) was obtained as an amorphous powder: ^1H NMR Table 1; IR (KBr) cm^{-1} 3600~2200, 3420 (m), 1740 (s), 1715 (s), 1620 (m), 1600 (w), 1590 (m).

Racemic 6-[1-(2-Methylbenzoyloxy)ethyl]-1-phenazinecarboxylic Acid (**19**)

The product prepared in analogous manner from 212 mg of racemic saphenic acid and 670 mg of freshly prepared *o*-toluic acid chloride was dissolved in 100 ml of $CHCl_3$ and washed with 2 N HCl, H_2O and satd NaCl. After drying (Na_2SO_4) and evaporation under reduced pressure 331 mg of a yellow product was obtained which was chromatographed on 80 g of silica gel impregnated with oxalic acid ($CHCl_3$ as the eluent). By recrystallization of the major product from acetone - hexane 113 mg (37%) of yellow prisms were obtained: MP 197~198°C; ^1H NMR Table 1; IR (KBr) cm^{-1} 3600~2200 (br), 3420 (m), 1740 (s), 1715 (s), 1620 (m), 1600 (m), 1570 (m); EI-MS *m/z* (relative intensity) 386 (12, M^+).

Racemic 6-[1-(2-Hydroxybenzoyloxy)ethyl]-1-phenazinecarboxylic Acid (**20**)

A mixture of 2.1 g of salicylic acid and 2 ml of thionyl chloride was refluxed for 2 hours. Excess reagent was removed under reduced pressure and the residue distilled at 70°C (0.07 Torr). The colorless oil (1.3 g) crystallized at *ca.* −4°C: EI-MS *m/z* (relative intensity) 361 (6, $C_{21}H_{13}ClO_6$ −35), 360 (6), 276 (13, $C_{14}H_9ClO_4$), 242 (18), 241 (100), 240 (38), 156 (11, $C_7H_5ClO_2$), 122 (40), 121 (95), 120 (98), 119 (22), 93 (47), 92 (52), 65 (70), 53 (11), 39 (38), 38 (12), 36 (23). The freshly distilled mixture

(500 mg) was reacted with racemic saphenic acid (211 mg) and 4-dimethylaminopyridine (120 mg) in 10 ml of abs $CHCl_3$ (16 hours, room temp). After dilution with 50 ml of $CHCl_3$ and washing with 2 N HCl, H_2O and satd NaCl the solution was dried and evaporated under reduced pressure. The yellow product was chromatographed on 80 g of silica gel impregnated with oxalic acid ($CHCl_3$, flash method). The homogeneous fractions (TLC) were collected, washed twice with water and, after drying (Na_2SO_4) and evaporation, chromatographed on Sephadex LH-20 (acetone). Recrystallization from acetone - hexane gave 93 mg of **20**, 29%, yellow prisms: MP 151~152°C; ^1H NMR Table 1; IR (KBr) cm^{-1} 3430 (br), 1740 (s), 1670 (s), 1615 (m), 1600 (s), 1570 (m); EI-MS *m/z* (relative intensity) 388 (2.4, M^+).

Anal Calcd for $C_{22}H_{16}N_2O_5$: C 68.04, H 4.15, N 7.21.
Found: C 67.86, H 3.87, N 6.99.

Racemic 6-[1-(2-Methoxy-6-methylbenzoyloxy)ethyl]-1-phenazinecarboxylic Acid (**21**)

2-Methoxy-6-methylbenzoic acid chloride (300 mg), freshly prepared from the acid[9] and $SOCl_2$, was added to a solution of 200 mg racemic saphenic acid and 300 mg of 4-dimethylaminopyridine in 10 ml of abs $CHCl_3$ and the mixture stirred 20 hours at room temperature. After dilution with 50 ml of $CHCl_3$ the solution was washed with 5% H_2SO_4 and evaporated under reduced pressure. The crude product (375 mg) was chromatographed twice on silica gel impregnated with oxalic acid ($CHCl_3$), the homogeneous (TLC) fractions washed with H_2O, dried and evaporated. Recrystallization from acetone - hexane 97 mg (31%) gave pure **21** as yellow crystals: MP 195~196°C; ^1H NMR Table 1; EI-MS *m/z* (relative intensity) 416 (3, M^+).

Anal Calcd for $C_{24}H_{20}N_2O_5$: C 69.22, H 4.84, N 6.73.
Found: C 68.97, H 4.71, N 6.65.

The methyl ester **22** prepared from **21** (6.3 mg) in 2 ml of $CHCl_3$ and 2 ml of CH_2N_2 (3% in ether, 20 hours, 4°C) was purified by preparative TLC (Silica gel Merck F_{254}, hexane - acetone, 2:1). Rf and ^1H NMR of the product (5.3 mg), were indistinguishable from those of a sample prepared from natural saphenamycin with CH_2N_2.

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References

1) GEIGER, A.; W. KELLER-SCHIERLEIN, M. BRANDL & H. ZÄHNER: Metabolites of microorganisms. 247. Phenazines from *Streptomyces antibioticus*, strain Tü 2706. J. Antibiotics 41: 1542~1551, 1988
2) KITAHARA, M.; H. NAKAMURA, Y. MATSUDA, M. HAMADA, H. NAGANAWA, K. MAEDA, H. UMEZAWA & Y. IITAKA: Saphenamycin, a novel antibiotic from a strain of *Streptomyces*. J. Antibiotics 35: 1412~1414, 1982
3) WOHL, A. & W. AUE: Ueber die Einwirkung von Nitrobenzol auf Anilin bei Gegenwart von Alkali. Ber. Dtsch. Chem. Ges. 34: 2442~2450, 1901
4) BIRKOFER, L. & A. WIDMANN: Neue Phenazin-Derivate und ihre tuberkulostatische Wirkung. Chem. Ber. 86: 1295~1302, 1953
5) PACHTER, I. J. & M. C. KLOETZEL: The Wohl-Aue reaction. I. Structure of benzo[a]phenazine oxides and syntheses of 1,6-dimethoxyphenazine and 1,6-dichlorophenazine. J. Am. Chem. Soc. 73: 4958~4961, 1951
6) TAKAHASHI, K.; I. TAKAHASHI, M. MORIMOTO & F. TOMITA: DC-86-M, a novel antitumor antibiotic. II. Structure determination and biological activities. J. Antibiotics 39: 624~628, 1986
7) ASANO, K.; K. TAKAHASHI, F. TOMITA & I. KAWAMOTO: DC-86-M, a novel antitumor antibiotic. I. Taxonomy of producing organism and fermentation. J. Antibiotics 39: 619~623, 1986
8) ZÄHNER, H.; H. DIDDENS & M. BRANDL: Biological activities of compounds related to saphenamycin. J. Antibiotics, in preparation
9) HAUSER, F. M. & S. A. POGANY: 2-Hydroxy-6-methylbenzoic acid derivatives. Synthesis 12: 814~815 1980