AN ENZYME INHIBITOR, PANOSIALIN, PRODUCED BY *STREPTOMYCES*. II

CHEMISTRY OF PANOSIALIN, 5-ALKYLBENZENE-1,3-DISULFATES

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Panosialin (I), an enzyme inhibitor, has been shown to be a mixture of 5-alkylbenzene-1,3-disulfates. The structures of the three major components are 5-isopentadecylbenzene-1,3-disulfate (Ia), 5-*n*-pentadecylbenzene-1,3-disulfate (Ib), and 5-isohexadecylbenzene-1,3-disulfate (Ic). The 5-*n*-alkyl homologs including Ib have been synthesized.

Panosialin (I) is a product of *Streptomyces* exhibiting inhibition of sialidase, acid phosphatase, and polygalacturonase as reported in another paper[1]. This paper is concerned with the structure elucidation of this new inhibitor.

Panosialin (I) is a sulfur-containing compound which is stable to alkali but less stable to acid. These properties and the presence of strong absorption bands at 1200~1300 cm^{-1} in its ir spectrum indicated the presence of a sulfate ester group in panosialin. Mild acid hydrolysis of I afforded sulfuric acid and compound II. The ir spectrum of II showed hydroxyl and aromatic absorption bands. Its methyl ether and acetate were prepared. The ratio (2 : 1) of the intensities of the O-methyl signal (nmr) *vs.* the aromatic hydrogen signal in the methyl ether as well as the same ratio of the acetyl methyl signal *vs.* the aromatic hydrogen signal in the ester suggested that II was a monoalkylated dihydric phenol. A positive mercuric nitrate test[2] suggested it was an alkyl resorcinol. This conclusion was supported by its uv[3~6] and ir spectra and moreover these spectral data indicated substitution at the 1,3,5 positions. Comparison of the nmr spectra of II, its acetate and methyl ether with those of orcinol[4] provided a convincing evidence that II was a 5-alkylresorcinol. The resistance of II to catalytic hydrogenation and the absence of olefinic hydrogen signals in its nmr

Fig. 1. Gas-chromatogram of compound III.

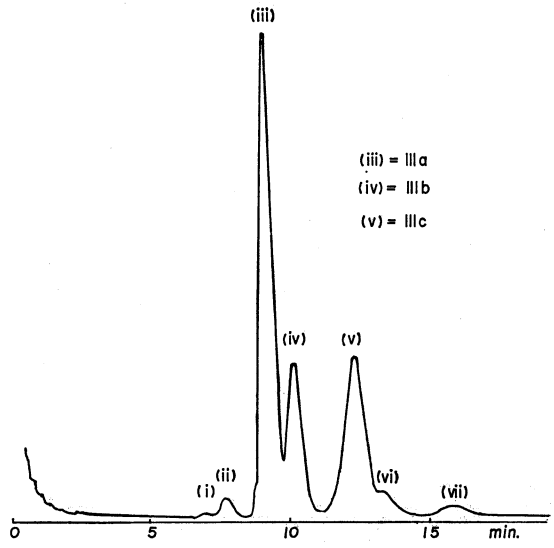
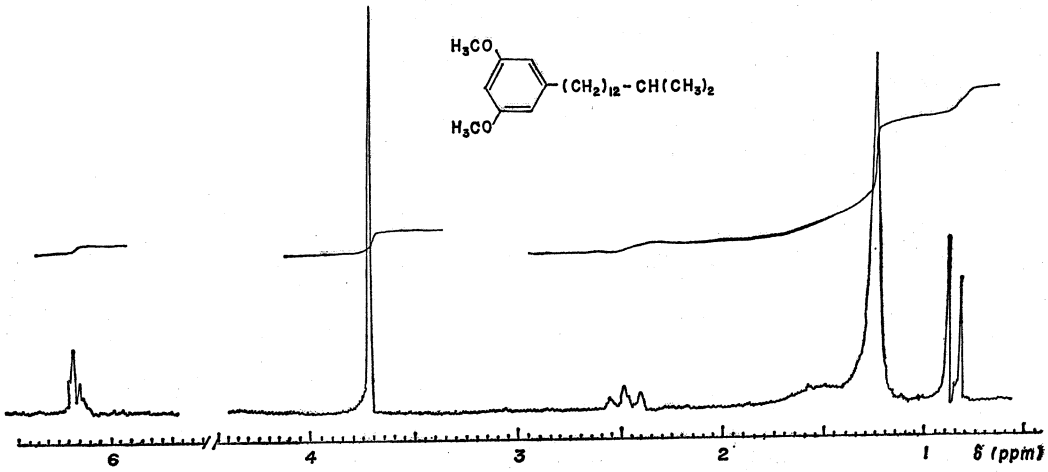


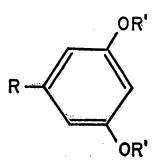
Fig. 2. 100 MHz nmr spectrum of compound IIIa in carbon tetrachloride with TMS as an internal standard.



spectrum indicated that the alkyl side chain was saturated.

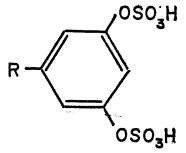
The heterogeneity of II was shown by its mass spectrum: II was a mitxture of the homologs, containing $C_{21}H_{36}O_2$ and $C_{22}H_{38}O_2$ as the main components in the ratio of 4.5 : 1 and other minor components. The gas chromatography of the dimethyl ether (III) of II revealed the presence of seven constituents as shown in Fig. 1. All constituents showed the maxima at 273 and 280 mμ[3~6]. Thus, they were concluded to be resorcinol dimethyl ethers with different alkyl side chains at the 5-positions.

The three major components, IIIa, IIIb, and IIIc were isolated by preparative gas chromatography, and the structures of these substances were deduced on the basis of the spectral data. The mass spectra showed the molecular formulas of IIIa, IIIb, and IIIc to be $C_{23}H_{40}O_2$, $C_{23}H_{40}O_2$, and $C_{24}H_{42}O_2$, respectively. The nmr spectrum of IIIc was very similar to that of IIIa (Fig. 2), and showed a doublet signal at δ 0.86 p.p.m. (6H, J=6.0 Hz) due to the terminal isopropyl residue in the alkyl side chain. These results established the structures of IIIa and IIIc to be the dimethyl ether of 5-isopentadecylresorcinol and that of 5-isohexadecylresorcinol, respectively. On the other hand, compound IIIb, an isomer of IIIa, was shown to be identical in all respects with the dimethyl ether of 5-*n*-pentadecylresorcinol which was obtained synthetically[6,7]. Therefore, compound II is a mixture of 5-isopentadecylresorcinol (IIa), 5-*n*-pentadecylresorcinol (IIb), 5-isohexadecylresorcinol (IIc), and four minor 5-alkylresorcinols. The rigorous structure determination of the four minor components, however, has not yet been completed. So far as we reviewed the literature, IIa and IIc have not been previously recorded among natural products, though IIb has been isolated from the plants[3,6].



IIa,	$R=(CH_2)_{12}$-$CH(CH_3)_2$;	R′=H	IIIa,	$R=(CH_2)_{12}$-$CH(CH_3)_2$;	R′=Me
b,	$R=(CH_2)_{14}$-CH_3;	R′=H	b,	$R=(CH_2)_{14}$-CH_3;	R′=Me
c,	$R=(CH_2)_{13}$-$CH(CH_3)_2$;	R′=H	c,	$R=(CH_2)_{13}$-$CH(CH_3)_2$;	R′=Me

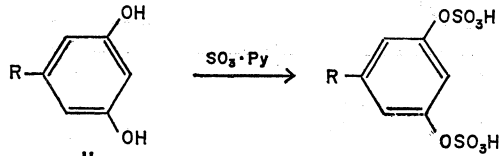
As already mentioned above, panosialin (I) yielded II and sulfuric acid on mild acid hydrolysis, indicating that I was a mixture of sulfated resorcinols with different alkyl side chains at the 5-positions. The molar ratio (1 : 1) of sulfur *vs.* potassium in the potassium salt of I and the absence of hydroxyl absorption bands in its ir spectrum suggested the following structure (I) for panosialin (I).



I

Ia, $R=(CH_2)_{12}-CH(CH_3)_2$
b, $R=(CH_2)_{14}-CH_3$
c, $R=(CH_2)_{13}-CH(CH_3)_2$
d, $R=(CH_2)_{12}-CH_3$
e, $R=(CH_2)_{18}-CH_3$

In order to confirm the structure, panosialin (I) was successfully prepared by the reaction of II with sulfur trioxide-pyridine[8].



The potassium salt of this synthetic material was identical with that of natural panosialin in all respects such as uv, ir, and nmr spectra, and the biological activity. Thus, panosialin (I) was concluded to be a mixture of seven homologs: three major components of 5-isopentadecylbenzene-1,3-disulfate (**Ia**), 5-*n*-pentadecylbenzene-1,3-disulfate (**Ib**), and 5-isohexadecylbenzene-1,3-disulfate (**Ic**), and other minor 5-alkylbenzene-1,3-disufates.

In addition to the structure determination of panosialin, it was of interest to synthesize some other homologs from the viewpoint of structure-activity relationship.

Treatment of 5-alkylresorcinols with excess sulfur trioxide-pyridine in anhydrous pyridine at room temperature, followed by neutralization with potassium hydroxide, yielded the desired products, dipotassium 5-alkylbenzene-1,3-disulfates (**Ib**, **Id**, and **Ie**), in good yields. Their uv and ir spectra were very similar to those of the potassium salt of I. The biological activity of **Ib**, one of the three major components of panosialin, was found to be approximately equal to that of panosialin as described in the preceding paper[1].

Experimental

General: Melting points were determined with a Yamato MT-I melting point apparatus, and are uncorrected. Rotations were determined with a Carl Zeiss 0.005° photoelectric polarimeter. Ir spectra were taken on a Hitachi Model EPI-S2 spectrometer. Uv spectra were determined on a Hitachi Model 124 spectrometer. Nmr spectra were taken on a Varian HA-100 spectrometer with TMS as internal standard. High resolution mass spectra were measured on a JEOL-JM-01SG spectrometer.

Potassium salt of panosialin (I): An analytical sample, m.p. 250.5~261.5° (dec.), was recrystallized twice from 1% aqueous potassium chloride solution.

Anal. Calcd. for $C_{21}H_{34}O_8S_2K_2$: C 45.30, H 6.16, O 22.99, S 11.51, K 14.05.
Calcd. for $C_{22}H_{36}O_8S_2K_2$: C 46.29, H 6.36, O 22.42, S 11.23, K 13.70.
Found: C 45.54, H 6.15, O 22.95, S 11.04, K 13.55.

Acid hydrolysis of panosialin: A 280-mg sample of the potassium salt of panosialin (I) was dissolved in 20 ml of hot water. To the solution was added 0.4 ml of 1 N hydrochloric acid and the mixture was heated at 50°C for 30 hours. After cooling, the mixture was extracted with four 25 ml portions of ether. The ether extract was washed with 20 ml of water, dried over anhydrous sodium sulfate, and filtered. The filtrate was evaporated under reduced pressure to give a colorless crystalline material. Recrystallization from hexane afforded 140 mg of **II** as colorless crystals. This material showed the following properties: m.p. 60°C; $[\alpha]_D^{25}$ 0° (*c* 1, MeOH); uv max (MeOH) 281 ($E_{1cm}^{1\%}$ 49.0) and 276 mμ ($E_{1cm}^{1\%}$ 50.0); ir (KBr) 3400 (OH), 1598 and 1470 cm^{-1} (aromatic ring); nmr ($CDCl_3$) δ 6.16~6.26 (3H, m, Ar-H), 4.86 (2H, s, Ar-OH) disappearing on exchange with deuterium oxide, 2.42~2.57 (2H, m, Ar-CH_2-), 1.28 (*ca.* 30H, -CH_2-), and 0.84~0.90 p.p.m. (5-6H, m, -CH_3).

Anal. Calcd. for $C_{21}H_{36}O_2$: C 78.69, H 11.32, O 9.98.
Calcd. for $C_{22}H_{38}O_2$: C 78.98, H 11.45, O 9.57.
Found: C 78.98, H 11.16, O 10.42.

To the aqueous phase described above was added 0.5 ml of 1 N hydrochloric acid. Immediately after the mixture was boiled for 3 minutes, 2 ml of a 10% barium chloride solution was added. The precipitate was transferred to a Gooch crucible, heated for 1 hour at 300°C, and ignited for 1 hour at 600°C. There was obtained 223 mg (0.96 mmole) of barium sulfate.

Dimethyl ether (III) of II: Compound **II** (250 mg) and methyl iodide (4 ml) were added to a mixture of dry acetone (25 ml) and anhydrous potassium carbonate (1.25 g), and the mixture was refluxed for four days. The mixture was cooled and filtered, and the solvent was eliminated from the filtrate, leaving a pale yellow oil. This material was dissolved in hexane and chromatographed on a column of Silica Gel (5 g). The column was eluted with hexane and the eluates were evaporated under reduced pressure. There was obtained 200 mg of the dimethyl ether (**III**) as a colorless oil: uv max (MeOH) 280 and 273 mμ; nmr (CCl_4) δ 6.10~6.19 (3H, m, Ar-H) and 3.71 p.p.m. (6H, s, OMe).

Diacetate of II: A 100-mg sample of **II** was dissolved in a mixture of pyridine (1 ml) and acetic anhydride (1 ml), and this solution was allowed to stand at room temperature overnight. The resulting clear solution was poured into ice-water (5 ml), and extracted with two 25 ml portions of chloroform. The chloroform extract was washed with water and dried over anhydrous sodium sulfate. The solvent was eliminated from the dried extract, leaving a colorless solid. This material was recrystallized from water-methanol, giving the diacetate as colorless crystals (70 mg): m.p. 31~33°C; uv max 261 mμ ($E_{1cm}^{1\%}$ 6.1); ir (KBr) 1775 cm^{-1} (phenolic acetate); nmr ($CDCl_3$) δ 6.73~6.77 (3H, m, Ar-H) and 2.27 p.p.m. (6H, s, OAc).

Attempted reduction of II: A solution of 50 mg of **II** in 20 ml of methanol was hydrogenated in the presence of 10 mg of 5% palladium on charcoal at room temperature and an initial pressure of 60 p.s.i. After 5 hours, the mixture was filtered, and the solvent was removed from the filtrate, leaving a colorless solid (48 mg). This material was shown to be identical with **II** (ir; nmr; uv; and tlc).

Gas chromatography of III; Qualitative analysis: The instrument used for the analysis was a Hitachi Model 063 equipped with a hydrogen flame ionization detector. The 2 m long column of 2 mm internal diameter was packed with 10% SE-30 supported on Chromosorb W, and was maintained at 235°C. The nitrogen flow rate was 100 ml/min. The relative retention times and per cent of total area of the seven components of **III** (Fig. 1) were as follows: (i) 0.69, 0.5%; (ii) 0.76, 1%; (iii)=**IIIa** 0.90, 47%; (iv)=**IIIb** 1.00, 19%; (v)=**IIIc** 1.21, 26.5%; (vi) 1.32, 4%; (vii) 1.56, 2%.

Preparative separation: Compound **III** was chromatographed on a Varian Aerograph Model 90-P3, using a thermal conductivity detector. The 152.5 cm (5 ft) long column of 0.88 cm (1/4 in) external diameter was packed with 5% SE-30 supported on Chromosorb

W, and it was maintained at 225°C. The helium flow rate was 60 ml/min. Using these conditions, a sample of 180 mg of compound **III** was separated into seven fractions, each of which was rechromatographed four times leading finally to seven components (the numbering corresponds to that used above): (i) <1 mg of a syrup, uv max (MeOH) 280 and 273 mμ; (ii) <1 mg of a syrup, uv max (MeOH) 280 and 273 mμ; (iii)=**IIIa** 26 mg of a syrup, uv max (MeOH) 280 and 273 mμ, nmr (CCl_4) δ 6.10~6.19 (3H, m, Ar-H), 3.71 (6H, s, OMe), 2.42~2.56 (2H, m, Ar-CH_2-) and 0.86 p.p.m. (6H, d, J=6.0 Hz, i-Pr methyls), mass *m/e* 348 (M^+, $C_{23}H_{40}O_2$); (iv)=**IIIb** 5 mg of solid identified as the dimethyl ether of *n*-pentadecylresorcinol which was prepared by the method of RIDLLY *et al.*[6]; (v)=**IIIc** 12 mg of a syrup, uv max (MeOH) 280 and 273 mμ, nmr (CCl_4) δ 6.09~6.19 (3H, m, Ar-H), 3.72 (6H, s, OMe), 2.42~2.57 (2H, m, Ar-CH_2-) and 0.86 p.p.m. (6H, d, J=6.0 Hz, i-Pr methyls), mass *m/e* 362 (M^+, $C_{24}H_{42}O_2$); (vi) <1 mg of a solid, uv max (MeOH) 280 and 273 mμ; (vii) <1 mg of a syrup, uv max 280 and 273 mμ.

Partial synthesis of panosialin (I) from compound II: A mixture of compound **II** (80 mg) and sulfur trioxide-pyridine[8] (130 mg) in 1.5 ml of anhydrous pyridine was stirred at room temperature for 24 hours. The resulting clear solution was poured into 15 ml of cold 0.1 N potassium hydroxide solution, forming a white precipitate. After the mixture had been allowed to stand in a refrigerator overnight, the product was collected by centrifugation, washed twice with cold water, once with ethanol, and then dried *in vacuo* over phosphorus pentoxide. There was obtained 97 mg of partly crystalline solid. Recrystallization from 1% aqueous potassium chloride solution gave 91 mg of colorless crystals, whose ir (KBr) and nmr (DMSO-d_6) spectra and mobilities on tlc were identical with those of the potassium salt of natural panosialin.

Dipotassium 5-*n*-tridecylbenzene-1,3-disulfate (**Id**): A mixture of *n*-tridecylresorcinol[6] (301 mg, 0.98 mmole) and sulfur trioxide-pyridine (555 mg, 3.47 mmoles) in 7 ml of anhydrous pyridine was stirred at room temperature for 24 hours. The resulting clear solution was poured into an ice-cooled solution of 0.1 N potassium hydroxide (69 ml), and the mixture was evaporated under reduced pressure to a volume of about 15 ml. The concentrate was kept at 0°C for 1 hour, and the resulting precipitate was collected by filtration, washed twice with cold water and once with ethanol, then dried *in vacuo* over phosphorus pentoxide. There was obtained 393 mg (76% yield) of **Id** as colorless crystals: m.p. 256~258°C (dec.); ir (KBr) 1222 cm^{-1} (S=O); nmr (DMSO-d_6) δ 6.70~6.89 (3H, m, Ar-H) and 0.80~0.94 p.p.m. (3H, m, -CH_3); uv max (1% aq. sodium chloride) 267 mμ (ε 389).

Anal. Calcd. for $C_{19}H_{30}O_8S_2K_2$: C 43.15, H 5.71, O 24.20, S 12.12.
Found: C 43.34, H 5.84, O 23.27, S 11.37.

Dipotassium 5-*n*-pentadecylbenzene-1, 3-disulfate (**Ib**): *n*-Pentadecylresorcinol[6] (150 mg, 0.47 mmole), sulfur trioxide-pyridine (245 mg, 1.5 mmoles), and anhydrous pyridine (14 ml) were mixed, and the products were worked up as described in the preparation of **I**, to give 211 mg (81% yield) of **Ib**, tlc of which revealed only one spot (Rf 0.4)*; m.p. 258.5~260°C (dec.); uv max (1% aq. sodium chloride) 263 mμ (ε 395).

Anal. Calcd. for $C_{21}H_{34}O_8S_2K_2$: C 45.29, H 6.15, O 22.98, S 11.51.
Found: C 45.32, H 5.92, O 22.91, S 10.84.

Dipotassium 5-*n*-nonadecylbenzene-1,3-disulfate (**Ie**): *n*-Octadecylresorcinol[4] (715 mg, 1.9 mmoles), sulfur trioxide-pyridine (994 mg, 6.2 mmoles), and anhydrous pyridine (14 ml) were mixed, and the product was worked up as described in the preparation of **I**, to give 998 mg (86% yield) of **Ie**: m.p. 255.5~258.5°C (dec.); uv max (1% aq. sodium chloride) 266 mμ (ε 380).

Anal. Calcd. for $C_{25}H_{42}O_8S_2K_2$: C 48.98, H 6.90, O 20.88, S 10.46.
Found: C 48.93, H 6.80, O 20.96, S 9.99.

* Tlc was performed with 0.25 mm thick Silica Gel F_{254} (E. Merck, Darmstadt). The tlc plates were developed with 4 : 1 (v/v) ethyl acetate-methanol and the spots were visualized with anisaldehyde sulfuric acid reagent or uv light.

References

1) Aoyagi, T.; M. Yagisawa, M. Kumagai, M. Hamada, Y. Okami, T. Takeuchi & H. Umezawa : An enzyme inhibitor, panosialin, produced by *Streptomyces*. I. Biological activity, isolation and characterization of panosialin. J. Antibiotics 24 : 860～869, 1971
2) Butenandt, A. & F. H. Stodola : Zur Kenntnis von α-und β-Leprosol. Ann. Chem. 539 : 40～56, 1939
3) Occolowitz, J. L. & A. S. Wright : 5-(10-Pentadecenyl)resorcinol from *Grevilla pyramidalis*. Australian J. Chem. 15 : 858～861, 1962
4) Wenkert, E.; E. M. Loeser, S. N. Mahapatra, F. Schenker & E. M. Wilson : Wheat bran phenols. J. Org. Chem. 29 : 435～439, 1964
5) Ritchie, E.; W. C. Taylor & S.T.K. Varetin : Chemical studies of the Proteaceae. I. *Grevillea robusta* A. Cunn and *Orites excelsa* R. Br. Australian J. Chem. 18 : 2015～2020, 1965
6) Ridley, D. D.; E. Ritchie & W. C. Taylor : Chemical studies of the Proteaceae. II. Some further constituents of *Grevillea robusta* A. Cunn.; experiments on the synthesis of 5-*n*-tridecylresorcinol (grevillol) and related substances. Australian J. Chem. 21 : 2979～2988, 1968
7) Wasserman, D. & C. R. Dawson : Cashew nut shell liquid. III. The cardol component of indian cashew nut shell liquid with reference to the liquid's vesicant activity. J. Am. Chem. Soc. 70 : 3675～3679, 1948
8) Sisler, H. H. & L. F. Audrieth : Addition compounds of sulfur trioxide. In inorganic syntheses. edited by J. C. Bailar, Jr., Vol. 2, pp. 173～174, McGraw-Hill, New York, 1946