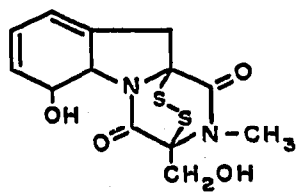
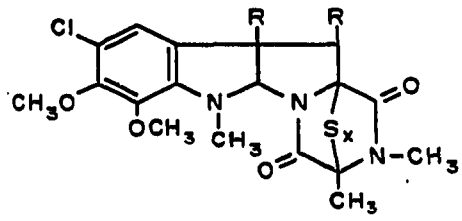
CHROM. 5013

Separation of polythiadioxopiperazine antibiotics by thin-layer chromatography

In recent years the number of 2,5-epipolythia-3,6-dioxopiperazine antibiotics has increased significantly due to isolation of new members of the series and by chemical transformations of the naturally-occurring compounds. By extensive chemical and/or X-ray crystallographic investigations the structure of gliotoxin[1] (I), sporidesmin[2,3] (II, X = 2, R = OH) and dehydrogliotoxin[4] (III, X = 2) was determined and other closely related compounds have since been isolated or synthesized[3,5]. More recently several compounds related to acetyl aranotin[6-8] (IV) have been described



(I)



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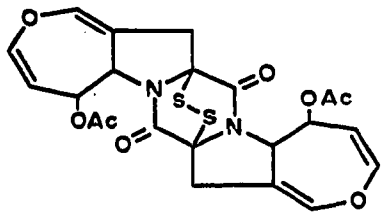

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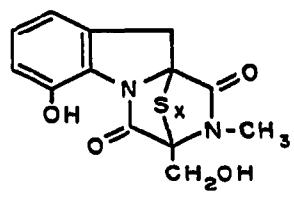

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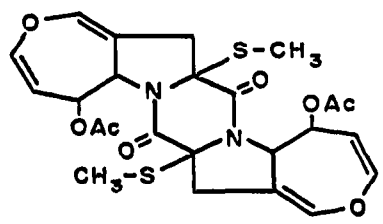

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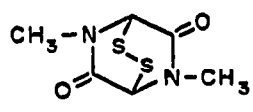

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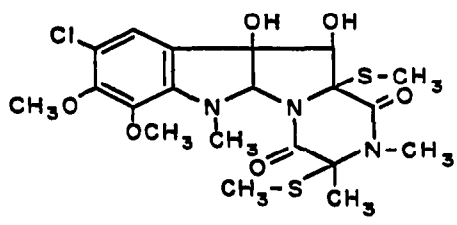

(VII)

(*i.e.* V[3,9]) and the synthesis of 1,4-dimethyl-2,5-piperazinedione (VI) has also been reported. Chetomin, another sulfur-containing natural product is also believed to contain the epipolythiadioxopiperazine moiety[10]. Most of the above compounds possess biological activity[10-12]. This communication deals with the thin-layer chromato-

* Issued as NRCC No. 11496.

graphic behavior of fifteen of these sulfur-containing compounds. The separation of polysulfides (II, X = 1, 2, 3, 4, R = OH) and (III, X = 1, 2, 3, 4) differing only in the number of sulfur atoms in the 2,5 bridge is particularly noted.

Compounds

Gliotoxin (I) was isolated from the fungus *P. terlikowskii*[4] and converted into dehydrogliotoxin (III, X = 2) as described[4]. Treatment of dehydrogliotoxin (III, X = 2) with triphenylphosphine[13] gave (III, X = 1) and with hydrogen polysulfide gave (III, X = 3) and (III, X = 4). Sporidesmin (II, X = 2, R = OH) was isolated from *P. chartarum* and reaction with triphenylphosphine gave (II, X = 1, R = OH)[3]. Sporidesmin E[14] (II, X = 3, R = OH), B (II, X = 2, R = H)[15] and D (VII)[16] were also isolated from the above fungus and the tetrasulfide (II, X = 4, R = OH) has also been isolated and prepared by treatment of (II, X = 2, R = OH) with hydrogen polysulfide[17]. Bisdethiodi(methylthio)-acetylaranotin (V) (BDA) and acetylaranotin (IV) were gifts of Eli Lilly Laboratories and the 1,4-dimethyl-2,5-piperazinedione (VI) was prepared by the method of TROWN[18]. Chetomin was obtained from *Chaetomium cochliodes*[11].

Chromatographic procedures

The solvent systems used and the R_F values for the compounds (I–VII) are given in Table I. Commercially available Merck Silica Gel F_{254} plates (20 × 20 cm, thickness 0.25 mm) were used and 5 μl of an 0.0002 % solution (in chloroform) was applied to the plate.

Detection

All compounds were visible under short wave UV light. When the chromato-

TABLE I

R_F VALUES FOR EPIPOLYTHIADIOXOPIPERAZINE ANTIBIOTICS

Compound	*Solvent system*				
	Benzene–ethyl acetate (4:1)	*Chloroform*	*Benzene–ether–acetic acid (70:30:1)*	*Hexane–tert.-butanol (9:1)*	*Chloroform–methanol (95:5)*
I	0.15	0.06	0.20	0.09	0.50
II, X = 1, R = OH	0.32	0.06	0.31	0.17	0.53
II, X = 2, R = OH	0.38	0.09	0.39	0.17	0.57
II, X = 3, R = OH	0.36	0.07	0.39	0.24	0.54
II, X = 4, R = OH	0.21	0.03	0.25	0.24	0.49
II, X = 2, R = H	0.39	0.11	0.41	0.23	0.59
III, X = 1	0.25	0.11	0.26	0.15	0.47
III, X = 2	0.40	0.22	0.44	0.26	0.58
III, X = 3	0.35	0.15	0.43	0.25	0.55
III, X = 4	0.19	0.05	0.23	0.17	0.49
IV	0.30	0.15	0.32	0.11	0.65
V	0.20	0.11	0.23	0.09	0.65
VI	0.16	0.13	0.22	0.07	0.56
VII	0.19	0.04	0.24	0.01	0.55
Chetomin	0.05	0.01	0.09	0.01	0.51

grams were sprayed with neutral aqueous 5 % silver nitrate solution spots of black (or brown) silver sulfide were obtained where the sulfur compounds were located.

The results obtained (Table I) show that by judicious choice of solvent systems separation of most of these sulfur-containing compounds can be obtained. In the dehydrogliotoxin series (III, X = 1–4) all four polysulfides as well as gliotoxin (I) are clearly separated with the solvent system benzene–ethyl acetate (4 : 1). The mono- and tetrasulfides of sporidesmin (II, X = 1 and 4, R = OH) have different R_F in benzene–ethyl acetate (4:1) (R_F 0.32 and 0.21) and in benzene–ether–acetic acid (70:30:1) and (R_F 0.31 and 0.25), from the chromatographically similar di- and trisulfides (II, X = 2 and 3, R = OH). Separation of the latter two compounds is readily effected in hexane–*tert.*-butanol (9:1). The above solvent systems have all been used for the separation of large quantities of material (100–300 mg) by preparative thin-layer chromatography. Sporidesmin B (II, X = 2, R = H) is generally less polar and sporidesmin D (VII) more polar than the other members of the series in most of the solvent systems used. Chetomin and (VI) are the two most polar compounds and both BDA (V) acetylaranotin (VI) are readily separable in all five solvent systems. With chloroform as solvent the R_F values are low for a single elution however with repeated elution (four times) excellent separations of most of the structurally-similar polysulfides is obtained.

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Received August 28th, 1970