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CYCLIC POLYSULPHIDES FROM *PARKIA SPECIOSA**

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Key Word Index—*Parkia speciosa*; Mimosaceae; cyclic polysulphides; lenthionine.

Abstract—Five polysulphides have been isolated from *Parkia speciosa*. The structures of four of these have been established as 1,2,4-trithiolane, 1,2,4,6-tetrathiepane, 1,2,3,5,6-pentathiepane (lenthionine) and 1,2,4,5,7,8-hexathionane. A further product has been tentatively assigned either the 1,2,4,6,7- or 1,2,4,5,7-pentathiocane structure.

INTRODUCTION

Cyclic polysulphides occur only rarely in nature and have only once previously been reported in higher plants. Lenthionine (1,2,3,5,6-pentathiepane) and the related compounds, 1,2,4,6-tetrathiepane and 1,2,4-trithiolane, which result from the enzymically induced cleavage of lentinic acid, have been isolated from the edible Shiitake mushroom of eastern Asia, *Lentinus edodes* [1]. These compounds have recently been found by Gmelin *et al.* [2] to occur in other Basidiomycetes mushrooms of the genus *Micromphale* and *Collybia* where they are considered to be formed from lentinic acid and epilentinic acid. Cyclic polysulphides of the lenthionine type have also been isolated from a red alga, *Chondria californica* [3], although the origin of these compounds remains unclear. Recently, Kameoka and Demizu [4] have identified a homologue of 1,2,4-trithiolane in the volatile oil from flowers and leaves of *Allium cepa*.

It is known that the root and germinating seedlings of many species of the Mimosaceae excrete substances possessing a pungent onion-like odour [5], which are organoleptically similar to that of lenthionine and related compounds (R. Gmelin and R. Susilo, unpublished observation). Such odour formation is especially noticeable when the endosperm of certain Mimosaceae species such as *Albizzia lophanta*, *Acacia farnesiana* or *Parkia speciosa* is treated with water.

It was originally considered [6] that the odorous compounds were related to the formation of unstable methanedithiol, a product (together with ammonia and pyruvate) of the action of CS lyase on djenkolic acid or *N*-acetyl-djenkolic acid. The present study, however, indicates that methanedithiol is only an intermediate in the formation of onion-like odours from *P. speciosa* (L.) Hassk. The seeds of this species either raw or cooked (locally called 'Peteh' or 'Petai' beans), are a favourite food in Indonesia where it is valued for its unique flavour. They have also been used in folk medicine because of their antibacterial effects on kidney, ureter and urinary bladder.

RESULTS AND DISCUSSION

Half-ripe, deep-frozen commercial *P. speciosa* seeds were crushed, incubated with water and the mixture extracted with chloroform. The concentrated extract was chromatographed on Si gel to yield three major fractions. Prep. TLC of these fractions afforded compounds **1**, **3**–**6**, all of which contained sulphur.

Compound **1** [0.56% crude extract, yellow oil, M^+ 233.87989 (obs.), calc. for $C_3H_6S_6$ 233.87941] exhibited a single peak in its ^1H NMR spectrum at δ 4.20, and was consequently assigned the symmetrical 1,2,4,5,7,8-hexathionane structure shown. Attempts to obtain a crystalline sample were unsuccessful. For example, attempted recrystallization from dioxan gave long, colourless crystals, mp 67–69°, identified (MS and NMR) as 1,2,4,5-tetrathiane, **2**.

Compounds **3** and **4** were identified as 1,2,4-trithiolane (0.12% yield) and lenthionine (0.07%) respectively by comparison with synthetic samples prepared according to Morita and Kobayashi [1].

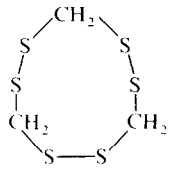
The pentasulphide **5** was isolated in 0.16% yield as huge colourless prisms, mp 102–104°. Its MS gave M^+ 201.90792 (calc. for $C_3H_6S_5$, 201.90725) and its ^1H NMR spectrum showed two singlet signals, at δ 4.25 (4 H) and 4.30 (2 H). The available data, however, made it impossible to distinguish between isomers **5a** and **5b**. Similar signals were also noted at δ 4.12 (4 H) and 4.25 (2 H) in the ^1H NMR spectrum of compound **6** (0.09%, mp 78–79°). The MS of this compound exhibited a molecular ion at *m/z* 170 and the spectrum proved identical with that of a sample of 1,2,4,6-tetrathiepane, synthesized by the method of Morita and Kobayashi [1].

Because of the evident biological activity of *P. speciosa* the antimicrobial effects of compounds **1** and **3** were examined against representative bacteria (both Gram-positive and Gram-negative) and against the fungus, *Candida albicans*. The results are presented in Table 1. Unfortunately, an insufficient sample was available for an investigation of the effect of **5**. It had previously been shown that **4** and **6** were effective against various bacteria and fungi [1].

EXPERIMENTAL

Mps are uncorrected. TLC and prep. TLC (0.5 mm) were performed on Si gel 60F254 Merck (Al_2O_3 sheets) and 60 PF254 Merck, respectively. Si gel 100 (70–230 mesh, Merck) was used for CC separations. Spray reagents used were 1% potassium iodoplatinate and 1% $KMnO_4$.

*Taken in part from the thesis of R. Susilo.



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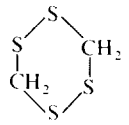

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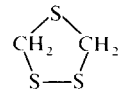

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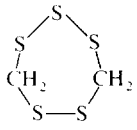

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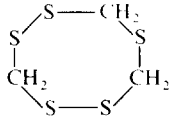

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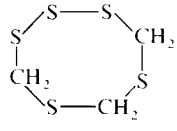

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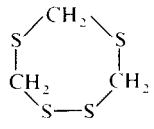

6

Table 1. Antimicrobial effects of compounds **1** and **3**

Test organism	**1**	**3**
Gram-positive bacteria:		
Staphylococcus aureus	+ +	+
Streptococcus faecium	+	+
Gram-negative bacteria:		
Escherichia coli	+	+ +
Klebsiella sp.	+ +	+ + +
Proteus mirabilis	+ +	+ + +
Pseudomonas aeruginosa	−	+ +
Fungus:		
Candida albicans	+ + +	+ +

+, Weak (< 2 mm circle of inhibition); + +, medium (2–5 mm circle of inhibition); + + +, strong (> 5 mm circle of inhibition).

Extraction. *P. speciosa* seeds (1 kg), half ripe and deep frozen were crushed for 5 min in a Waring blender. The solids were just covered with H_2O incubated for 3 hr at 45° and centrifuged. The residue was extracted with $CHCl_3$ (11 × 4) by stirring at room temp. for 30 min. The combined $CHCl_3$ phases were dried (Na_2SO_4) and evapd *in vacuo* (below 50°) to yield a dark oily residue (10 g). During all subsequent work, a temp. of 50° was not exceeded.

Chromatographic separation. The crude extract was chromatographed on Si gel (70 cm × 4 cm column) with C_6H_{14}–$CHCl_3$ (1:1) as eluant. Fractions of 18 ml were collected every 10 min. Fractions 20–32 were combined and evapd *in vacuo* to yield fraction 1 (4.5 g). This material was rechromatographed on a 65 cm × 3 cm column using C_6H_{14} and 12-ml fractions were collected each 10 min. Fractions 34–40, 41–50 and 58–71 were bulked to give fractions 1a/1, 1a/2 and 1b, respectively. Fraction 1a/1 possessed a strong typical odour of *Parkia* beans and yielded a yellow oil on concn. The product (56 mg, **1**) was homogeneous in three different TLC systems: R_f 0.15 C_6H_{14}, 0.20 cyclohexane, 0.52 $CHCl_3$–C_6H_{14} (2:3). ^{1}H NMR ($CDCl_3$): δ 4.20 (*s*); IR (liquid film) cm^{-1}: 2970, 2915, 1390, 1350, 1192, 1178, 1084, 835, 815, 724, 695; MS *m/z* (rel. int.): 234(1), 188(5), 170(1), 156(13), 142(10), 138(3), 124(100), 110(12), 96(2), 92(1), 78(85), 64(10), 60(16), 46(38), 45(67). Attempted recrystallization of the oil from dioxan gave a colourless product, mp 67–69°, which was homogeneous by TLC: R_f 0.14 C_6H_{14}, 0.19 cyclohexane, 0.52 $CHCl_3$–C_6H_{14} (2:3). The compound contained only four sulphur atoms MS: M^+ 155.9117 (obs.) calc. for $C_2H_4S_4$; 155.9193; *m/z* (rel. int.): 158(12), 156(69), 124(6), 112(7), 110(59), 78(21), 77(5), 76(7), 66(4), 64(38), 48(6), 47(6), 46(74), 45(100); ^{1}H NMR ($CDCl_3$): δ 4.0, (*br*).

Fraction 1a–2 contained traces of **1** as well as small amounts of two other compounds. Separation was effected by prep. TLC using multi-development with C_6H_{14}. Compound **3** (12 mg) was found to be 1,2,4-trithiolane. R_f: 0.14 C_6H_{14}, 0.18 cyclohexane, 0.51 $CHCl_3$ C_6H_{14}(2:3); ^{1}H NMR ($CDCl_3$): δ 4.17 (*s*); MS *m/z* (rel. int.): 124(100), 78(92), 64(4), 60(23), 59(14), 46(21), 45(21), 45(43). The second substance **4** had R_f 0.13 C_6H_{14}, 0.18 cyclohexane, 0.53 $CHCl_3$–C_6H_{14} (4:6) and yielded colourless crystals from dioxan, mp 59–61° (7 mg). ^{1}H NMR ($CDCl_3$): δ 4.32 (s); MS *m/z* (rel. int.): 188 (40), 156(7), 142(100), 124(60), 110(13), 96(6), 78(91), 64(21), 46(30), 45(49). Comparison with a synthetic sample showed **4** to be 1,2,3,5,6-pentathiepane (lenthionine).

Fraction 1b contained two components, **5** (major) and **6** (minor), which were separated by prep. TLC ($CHCl_3$–C_6H_{14}, 2:3) Crude **5** was dissolved in $CHCl_3$–C_6H_{14} (1:9) and chilled at +4°. After some days, long colourless prisms (16 mg) of pure **5** separated and these were filtered and washed with cold EtOH–C_6H_{14}, mp 102–104°. R_f 0.06 C_6H_{14}, 0.08 cyclohexane, 0.45 $CHCl_3$–C_6H_{14} (4:6); ^{1}H NMR ($CDCl_3$): δ 4.25 (4 H, *s*) 4.30 (2 H, *s*); MS *m/z* (rel. int.): 202 (0.4), 170(86), 156(1), 137(7), 124(72), 110(1), 78(100), 64(6), 60(35), 59(11), 46(33), 45(54). These data are consistent with either 1,2,4,5,7-pentathiocane (**5a**) or 1,2,3,5,7-pentathiocane (**5b**).

The minor compound **6** of fraction 1b was purified, after prep. TLC (above), by sublimation to give colourless crystals (9 mg) mp 78–79°. R_f 0.06 C_6H_{14}, 0.08 cyclohexane, 0.48 $CHCl_3$–C_6H_{14} (2:3); ^{1}H NMR ($CDCl_3$): δ 4.21 (4 H, *s*), 4.25 (2 H, *s*); MS *m/z*, (rel. int.): 170(60), 137(6), 124(60), 105(4), 93(10), 78(100), 64(11),

60(35), 46(47), 45(70). Comparison with a synthetic sample confirmed the identity of **6** as 1,2,4,6-tetrathiepane.

Antibiotic activity. Activities were determined using the filter paper test. Samples (800 μg) were applied to discs of MN 827 (dia 6 mm, thickness 0.7 mm). Cultures were grown on DST agar test medium (Oxoid Co., pH 7.4) with standard 2 culture medium bouillon (Merck 7884). Incubation was routinely carried out at 27° for 2 days. Control substances penicillin G-sodium (100 I.E.) and gentamycin (10 μg) were tested against the test strains, and Nystatin (100 I.E.) was tested against *Candida albicans*. Activity was assessed from the degree of inhibition (Table 1).

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