Synthesis of Various Nucleosides Derived from Benzothiazole-2-thione as Potential Antifungal Agents

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By the catalyzed fusion procedure, benzothiazole-2-thione was glycosylated with various peracetylated derivatives of D-arabinose, D-xylose, and D-glucose. With D-xylopyranose and D-arabinofuranose derivatives, anomeric mixtures were obtained in which the *trans-N*-glycoside predominated. In all other cases the trans anomer was the only product characterized. The structures of all new compounds have been rigorously established from their physicochemical properties. None of the studied glycosides inhibit the in vitro growth of *Fusarium oxysporum* f. sp. *albedinis*.

This paper describes the synthesis of some nucleosides potentially active against *Fusarium oxysporum* f. sp. *albedinis*, which causes the white illness (Bayoud) of the palm tree.

In previous works (Gosselin et al., 1978, 1979), the *N*-β-D-ribofuranosyl nucleosides of some azole-2-thiones were obtained. These nucleosides and their aglycons, namely the imidazole, benzimidazole, benzoxazole, and benzothiazole-2-thione were tested in vitro against *F. oxysporum* f. sp. *albedinis* (Bounaga, 1980). Only benzothiazole-2-thione showed any antifungal activity; this activity seemed connected with the presence of the group NC(—S)=S found in dithiocarbamic acids, known for their remarkable antifungal properties (Togerson, 1969). Surprisingly, a loss of fungitoxicity after ribosylation of benzothiazole-2-thione was observed. Such different activity between the aglycon and its riboside prompted us to study the possible effect of the variation of the sugar moiety on the antifungal activity of benzothiazole-2-thione.

As possible vectors, we selected three sugars that are more or less well metabolized by the palm tree fusarium (Bounaga, 1976), namely D-xylose, D-arabinose, and D-glucose.

The *N*-glycopyranosyl nucleosides of benzothiazole-2-thione were synthesized by the iodine-catalyzed fusion reaction (Barascut et al., 1976). The anomerization of the starting peracetylated glycopyranosides **1–3** under the fusion reaction conditions was also investigated.

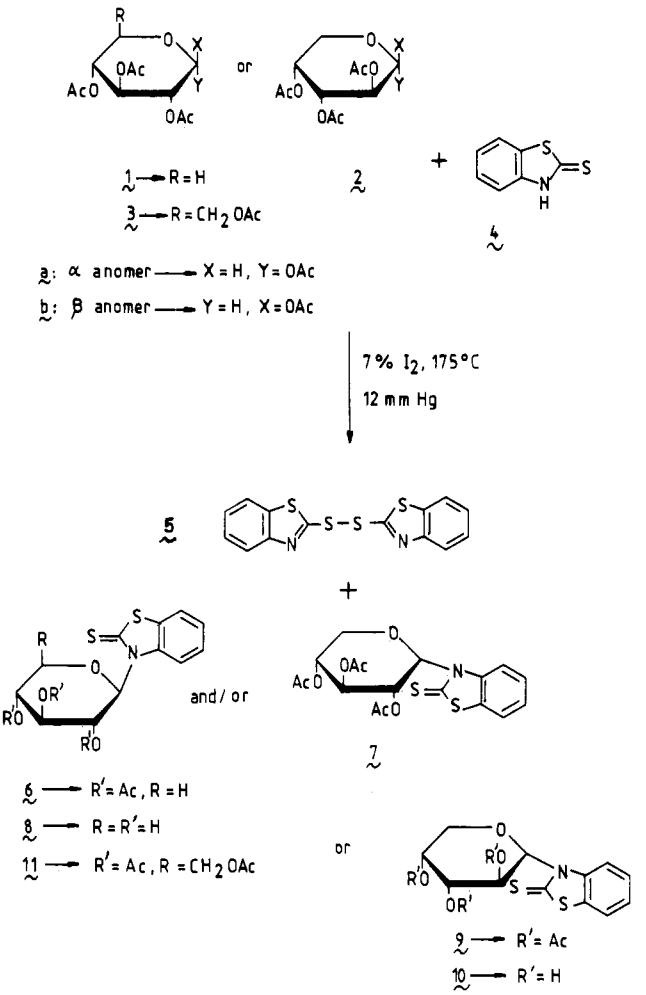
DISCUSSION

Each peracetylated α (**a**) and β (**b**) anomer of D-xylo- (**1**), D-arabino- (**2**), and D-glucopyranose (**3**) was heated at 175 °C in the presence of iodine. Analysis by ^{1}H NMR showed that only the sugars of β configuration (**1b**, **2b**, and **3b**) anomerized, leading to an α/β ratio of 1/3. This result proved that the peracetylated glycopyranosyl sugars of α configuration are thermodynamically more stable than their β anomers.

Initially separate condensations of benzothiazole-2-thione (**4**) with α and β anomers of peracetylated D-xylo- (**1**), D-arabino- (**2**), and D-glucopyranose (**3**) were carried out. For each series, TLC studies showed formation of the same products regardless of the anomeric configuration of the starting sugar. Column chromatography served to separate the products (Scheme I).

In the D-xylopyranose series chromatography of the oil obtained by condensation of **4** with **3b** led to isolation of

Scheme I



three compounds (**5–7**) in yields of 6.3, 25.6, and 7.9%, respectively.

The first of these compounds was identified as 2,2′-dithiobis(benzothiazole) (**5**) by its physicochemical properties (Koch, 1949; Gosselin et al., 1978). Derivatives **6** and **7** possessed nucleosidic structures as evidenced by the presence of a molecular ion at *m/z* 425 and by elemental analysis. Comparison of the UV spectra of these compounds with benzothiazole-2-thione substituted on the N-3 nitrogen (Gosselin et al., 1978) or on the exocyclic sulfur (Moore and Waight, 1952) atom showed that they were N-glycosylated products. The anomeric configurations were assigned from ^{1}H NMR spectral data. Consistent with established criteria (Nishimura and Shimizu, 1965; Stevens and Fletcher, 1968; Townsend, 1973) (the H-1′ of a 1′,2′-cis nucleoside resonates at a lower field than that of a 1′,2′-trans nucleoside) the β configuration was at-

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tributed to **6** (H-1′ at 6.80 ppm) and the α configuration to **7** (H-1′ at 7.00 ppm). For **6**, the coupling constant between H-1′ and H-2′ ($J_{1',2'}$ = 9.0 Hz) confirmed the β anomeric assignment, consistent with a trans-diaxial orientation of H-1′ and H-2′ (Lemieux and Lown, 1963; Lemieux and Stevens, 1965). Thus, the nucleosides **6** and **7** were characterized respectively as 3-(2′,3′,4′-tri-*O*-acetyl-β- and -α-D-xylopyranosyl)benzothiazole-2-thione. The acyl blocking groups of compound **6** were then catalytically removed with sodium methoxide in methanol to afford the desired β-D-xylopyranosyl nucleoside **8**, the structure of which was supported by the study of its physicochemical properties.

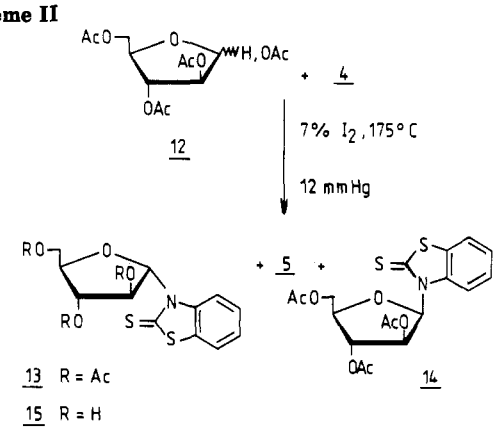
In the D-arabinopyranose series the fusion reaction of aglycon **4** with the α anomer of tetra-*O*-acetylarabinopyranose (**2a**) was carried out. Column chromatography of the crude reaction mixture allowed isolation of 2,2′-dithiobis(benzothiazole) (**5**) in 5% yield and a new compound (**9**) in 53% yield. This was identified as 3-(2′,3′,4′-tri-*O*-acetyl-α-D-arabinopyranosyl)benzothiazole-2-thione. Elemental analysis and mass spectrum indicated that it was a peracetylated nucleoside of benzothiazole-2-thione. Furthermore, its UV spectrum was comparable to that of N-substituted derivatives of this aglycon. Finally, in addition to protons corresponding to the aglycon, the ^{1}H NMR spectrum showed a doublet at 6.80 ppm assigned to the anomeric proton. The value of its coupling constant ($J_{1',2'}$ = 9.0 Hz) supported a trans-diaxial orientation for H-1′ and H-2′ and therefore an α anomeric configuration for compound **9**. Nucleoside **9** was then deacetylated with methanolic sodium methoxide to afford 3-α-D-arabinopyranosylbenzothiazole-2-thione (**10**).

The condensation of benzothiazole-2-thione (**4**) with the β anomer **3b** of pentacetylated D-glucopyranose led to 2,2′-dithiobis(benzothiazole) (**5**) (9% yield) as well as to two new compounds separated by column chromatography. The less polar derivative was isolated in too low a quantity for characterization. The other compound had a nucleosidic structure, as confirmed by elemental analysis and mass spectrum. Its UV spectrum showed that it was an *N*-nucleoside. Furthermore, its ^{1}H NMR spectrum showed the signal of the H-1′ proton as a doublet centered at 6.90 ppm, with a coupling constant $J_{1',2'}$ = 9.0 Hz. This value supported a trans-diaxial orientation for H-1′ and H-2′ and allowed assignment of the β configuration to this compound. Thus, this nucleoside was characterized as 3-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)benzothiazole-2-thione (**11**).

To complete the present work, we synthesized an *N*-D-pentofuranosylnucleoside of the benzothiazole-2-thione (**4**) to determine if a transition from the pyranose to the furanose structure could modify the antifungal properties. The sugar chosen was the less metabolized sugar by the *Fusarium*, namely D-arabinose. Tetra-*O*-acetyl-D-arabinofuranose (**12**) (Guthrie and Smith, 1968) was subjected to the iodine-catalyzed fusion reaction with benzothiazole-2-thione (**4**) under the same experimental conditions as those used during the synthesis of *N*-glycopyranosylnucleosides (Scheme II). Column chromatography of the crude reaction mixture afforded 2,2′-dithiobis(benzothiazole) (**5**) (9% yield) and two new compounds (**13** (36% yield) and **14** (3.8% yield)).

The nucleosidic structures of **13** and **14** were confirmed by their elemental analysis and mass spectrum. The site of glycosylation was established as N-3 from UV spectra. Their anomeric configurations were assigned from ^{1}H NMR spectral data. On the basis of the observed difference in the chemical shifts of the anomeric protons, we

Scheme II



ascribed the α configuration to **13** (H-1′ at 6.80 ppm) and the β configuration to **14** (H-1′ at 7.20 ppm). Deacetylation of **13** gave 3-α-D-arabinofuranosylbenzothiazole-2-thione (**15**).

BIOLOGICAL EVALUATION

The biological activity of compounds **6**, **8–10**, **13**, and **15** was evaluated against the in vitro growth of *F. oxysporum* f. sp. *albedinis*.

Unlike their aglycon **4**, none of these nucleosides exhibited antifungal activity; the weak inhibitor effect previously shown by 3-β-D-ribofuranosylbenzothiazole-2-thione (Bounaga, 1980) was not observed at concentrations as high as 1.5 mM for these new compounds.

Glycosylation of benzothiazole-2-thione (**4**) leads to a loss of in vitro antifungal activity in agreement with previous results (Bounaga, 1980); changes in the nature of the sugar and its cyclic form have no effect. It is possible however that the activity of these nucleosides might be different in vivo, as demonstrated for many therapeutic agents (Wain and Carter, 1977; Ryley et al., 1981).

EXPERIMENTAL SECTION

General Chemical Synthesis. Evaporation of solvents was done with a rotary evaporator under reduced pressure (water aspirator). Melting points were determined on a Buchi 510 apparatus and are uncorrected. Ultraviolet spectra (UV) were recorded on an Optica Model 10 spectrophotometer; numbers in parentheses are extinction coefficients ($\epsilon \times 10^{-3}$). Proton nuclear magnetic resonance (^{1}H NMR) were determined at ambient temperature on Varian EM 390 or HA 100 spectrometers: the chemical shifts are expressed in parts per million downfield from internal tetramethylsilane. The signals are described as s, singlet; d, doublet; t, triplet; m, multiplet; and br s, broad signal. The presence of exchangeable protons was confirmed by exchange with D_2O followed by reintegration. Mass spectra (MS) were measured with use of a JEOL JMSD 100 instrument, at 75 eV by direct-probe sample introduction. Elemental analyses were determined by the Microanalysis Service of the CNRS (Division of Montpellier). Thin-layer chromatography (TLC) was performed on precoated aluminum sheets with silica gel 60 F_{254} (Merck, Catalog No 5554), visualization of products in chromatograms being accomplished by UV absorbance followed by charring with 10% ethanolic sulfuric acid and heating. Column chromatography was performed with silica gel 60 (Merck, Catalog No. 9385). Other pertinent information and the solvent chromatography are given at their appropriate places in the individual experimental procedures.

Preparation of the Peracetylated Starting Sugars 1–3 and 12. These compounds were synthesized according to the literature data: **1a**, Durette and Horton (1971); **1b**,

Hudson and Johnson (1915); **2a** and **2b**, Kuszmann and Vargha (1962); **3a** and **3b**, Vogel (1956); **12**, Guthrie and Smith (1968).

General Procedure for the Fusion of Peracetylated Sugar with Benzothiazole-2-thione (4). A dry, finely powdered mixture of benzothiazole-2-thione (4) (0.012 mol), peracetylated sugar (0.012 mol), and twice-sublimed iodine (7% mol/mol of 4) were fused in a pear-shaped flask for 15 min with a vacuum of ca. 12 mmHg, at 175 °C. The crude reaction mixture was dissolved in methanol, treated with powdered animal charcoal, and then filtered through Celite. The filtrate was evaporated to dryness, dissolved in the minimal amount of chloroform, and applied to a silica gel column.

General Procedure for the Preparation of Unprotected Nucleosides 8, 10, and 15. The protected nucleosides **6**, **9**, and **13** were dissolved with stirring in a freshly prepared solution (ca. 10 mL/mmol of nucleoside) of 0.3 N sodium methoxide, made by adding appropriate quantity of small slivers of sodium to anhydrous methanol. When TLC indicated that the reaction was complete (ca. 4 h), the same volume of water was added and the solution was neutralized to pH 6–7 by the addition of resin Dowex 50WX2 (pyridinium form). After the resin was filtered and washed with warm methanol and water, the combined filtrates were evaporated to dryness. The residue was dissolved in water and repeatedly washed with chloroform and diethyl ether. The aqueous phase was then filtered and evaporated to dryness to afford the corresponding deblocked nucleosides **8**, **10**, and **15**.

3-(2′,3′,4′-Tri-*O*-acetyl-β-D-xylopyranosyl)benzothiazole-2-thione (6). The crude material obtained from the fusion of **4** with **1b** was chromatographed on a silica gel column with chloroform–ethyl acetate (95/5) to afford 1.02 g (25.6% yield) of compound **6**, which was crystallized from dichloromethane–petroleum ether: mp 195 °C; UV (MeOH) λ_{max} 328 nm (19.7), 240 (8.3), 229 (11.2); ^{1}H NMR ($CDCl_3$) δ 1.90 (s, 3 H, CH_3CO), 2.01 (s, 3 H, CH_3CO), 2.10 (s, 3 H, CH_3CO), 3.6–4.3 (m, 2 H, H-5′,5″), 5.4 (m, 1 H, H-4′), 5.5–5.8 (m, 2 H, H-2′ and 3′), 6.80 (d, 1 H, H-1′, $J_{1',2'}$ = 9.0 Hz), 7.2–7.8 (m, 4 H, H-4,5,6,7); MS, *m/z* (relative intensity) 425 ($M^{\bullet+}$, 30), 259 (58), 168 (51), 167 (45), 166 (23), 157 (74), 139 (94), 97 (100). Anal. Calcd for $C_{18}H_{19}NO_7S_2$: C, 50.82; H, 4.47; N, 3.29. Found: C, 50.8; H, 4.6; N, 3.2.

3-(2′,3′,4′-Tri-*O*-acetyl-α-D-xylopyranosyl)benzothiazole-2-thione (7). This nucleoside was obtained as slower eluted compound from the foregoing column chromatography, with a 7.9% yield (0.2 g). It was crystallized from dichloromethane–petroleum ether: mp 190 °C; UV (MeOH) λ_{max} 322 nm (26.9), 238 (15.0), 226 (18.7); ^{1}H NMR ($CDCl_3$) δ 1.60 (s, 3 H, CH_3CO), 2.20 (s, 2×3 H, 2 CH_3CO), 4.30 (s, 2 H, H-5′,5″), 4.80 (s, 1 H, H-4′), 5.1–5.4 (m, 2 H, H-2′ and 3′), 7.00 (d, 1 H, H-1′, $J_{1',2'}$ = 1.8 Hz), 7.2–8.2 (m, 4 H, H-4,5,6,7); MS, *m/z* (relative intensity) 425 ($M^{\bullet+}$, 24), 259 (43), 258 (14), 199 (31), 168 (30), 167 (26), 166 (13), 157 (54), 97 (100). Anal. Calcd for $C_{18}H_{19}NO_7S_2$: C, 50.82; H, 4.47; N, 3.29. Found: C, 50.7; H, 4.6; N, 3.2.

3-β-D-Xylopyranosylbenzothiazole-2-thione (8). After the general deblocking procedure was performed on 0.3 g (0.71 mmol) of **6**, the crude material obtained was precipitated from chloroform–methanol to give 0.15 g (71%) of pure **8**: mp 220 °C; UV (MeOH) λ_{max} 326 nm (13.7), 240 (7.3), 227 (8.0); ^{1}H NMR (Me_2SO-d_6) δ 3.2–4.2 (m, 5 H, H-2′,3′,4′,5′,5″), 5.2 (br s, 3 H, OH-2′,3′,4′), 6.30 (d, 1 H, H-1′, $J_{1',2'}$ = 9.0 Hz), 7.3–7.8 (m, 4 H, H-4,5,6,7); MS, *m/z* (relative intensity) 299 ($M^{\bullet+}$, 41), 168 (100), 167 (88), 166 (18), 135 (29), 132 (29). Anal. Calcd for $C_{12}H_{13}NO_4S_2$·0.25H_2O: C, 47.43; H, 4.48; N, 4.61. Found: C, 47.7; H, 4.2; N, 4.7.

3-(2′,3′,4′-Tri-*O*-acetyl-α-D-arabinopyranosyl)benzothiazole-2-thione (9). The crude material obtained from the fusion of **4** with **2a** was chromatographed on a silica gel column with chloroform–ethyl acetate (9/1) to afford 2.78 g (53% yield) of compound **9**, which was crystallized from dichloromethane–petroleum ether: mp 174 °C; UV (MeOH) λ_{max} 327 nm (28.4), 239 (9.2), 227 (16.0); ^{1}H NMR ($CDCl_3$) δ 1.90 (s, 3 H, CH_3CO), 2.00 (s, 3 H, CH_3CO), 2.30 (s, 3 H, CH_3CO), 3.9–4.3 (m, 2 H, H-5′,5″), 5.2–5.6 (m, 2 H, H-3′ and 4′), 5.9 (t, 1 H, H-2′), 6.80 (d, 1 H, H-1′, $J_{1',2'}$ = 9.0 Hz), 7.3–8.1 (m, 4 H, H-4,5,6,7); MS, *m/z* (relative intensity) 425 ($M^{\bullet+}$, 65), 259 (45), 168 (48), 167 (44), 166 (7), 157 (73), 139 (95), 97 (100). Anal. Calcd for $C_{18}H_{19}NO_7S_2$: C, 50.82; H, 4.47; N, 3.29. Found: C, 50.6; H, 4.6; N, 3.2.

3-α-D-Arabinopyranosylbenzothiazole-2-thione (10). After the general deblocking procedure was performed on 0.3 g (0.71 mmol) of **9**, the crude material obtained was precipitated from methanol–water to give 0.17 g (80 %) of pure **10**: mp 130 °C; UV (MeOH) λ_{max} 327 nm (19.3), 239 (9.2), 277 (10.9); ^{1}H NMR (Me_2SO-d_6) δ 3.2–4.0 (m, 4 H, H-3′,4′,5′,5″), 4.40 (t, 1 H, H-2′), 5.0–5.3 (br s, 3 H, OH-2′,3′,4′), 6.30 (d, 1 H, H-1′, $J_{1',2'}$ = 9.0 Hz), 7.3–8.2 (m, 4 H, H-4,5,6,7); MS, *m/z* (relative intensity) 299 ($M^{\bullet+}$, 30), 168 (100), 167 (90), 166 (19), 140 (30), 133 (18), 132 (22), 127 (40). Anal. Calcd for $C_{12}H_{13}NO_4S_2$·0.33H_2O: C, 46.29; H, 4.64; N, 4.50. Found: 46.4; H, 4.7; N, 4.4.

3-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)benzothiazole-2-thione (11). The crude material obtained from the fusion of **4** with **3b** was chromatographed on a silica gel column with chloroform–ethyl acetate (95/5) to afford 0.49 g (7.7% yield) of compound **11**, which was crystallized from dichloromethane–petroleum ether: mp 192 °C; UV (MeOH) λ_{max} 326 nm (21.2), 274 (8.3), 262 (9.8), 238 (16.4), 266 (22.5); ^{1}H NMR ($CDCl_3$) δ 1.90 (s, 3 H, CH_3CO), 2.00 (s, 3 H, CH_3CO), 2.05 (s, 3 H, CH_3CO), 2.10 (s, 3 H, CH_3CO), 4.0–4.3 (m, 3 H, H-5′,6′,6″), 5.2–5.8 (m, 3 H, H-2′,3′,4′), 6.90 (d, 1 H, H-1′, $J_{1',2'}$ = 9.0 Hz), 7.2–7.8 (m, 4 H, H-4,5,6,7); MS, *m/z* (relative intensity) 497 ($M^{\bullet+}$, 34), 330 (52), 169 (100), 167 (37), 109 (75). Anal. Calcd for $C_{21}H_{23}NO_9S_2$: C, 50.70; H, 4.62; N, 2.81. Found: C, 50.4; H, 4.6; N, 2.6.

3-(2′,3′,5′-Tri-*O*-acetyl-α-D-arabinofuranosyl)benzothiazole-2-thione (13). The crude material obtained from the fusion of **4** with **12** was chromatographed on a silica gel column with chloroform–ethyl acetate (8/2) to afford 1.9 g (36% yield) of compound **13**, which was crystallized from dichloromethane–petroleum ether: mp 170 °C; UV (EtOH) λ_{max} 329 nm (22.2), 240 (10.3), 229 (12.5); ^{1}H NMR (Me_2SO-d_6) δ 1.85 (s, 3 H, CH_3CO), 1.95 (s, 3 H, CH_3CO), 2.30 (s, 3 H, CH_3CO), 4.0–5.8 (m, 5 H, H-2′,3′,4′,5′,5″), 6.80 (d, 1 H, H-1′, $J_{1',2'}$ = 9.0 Hz), 6.4–7.9 (m, 4 H, H-4,5,6,7); MS, *m/z* (relative intensity) 425 ($M^{\bullet+}$, 17), 259 (76), 199 (13), 168 (31), 167 (28), 166 (17), 157 (31), 139 (100), 97 (71). Anal. Calcd for $C_{18}H_{19}NO_7S_2$: C, 50.82; H, 4.47; N, 3.29. Found: C, 50.5; H, 4.4; N, 3.3.

3-(2′,3′,5′-Tri-*O*-acetyl-β-D-arabinofuranosyl)benzothiazole-2-thione (14). This nucleoside was obtained as the slower eluting compound from the foregoing column chromatography, with a 3.8% yield (0.20 g) of a yellow syrup: UV (EtOH) λ_{max} 327 nm (18.2), 240 (10.9), 228 (13.4); ^{1}H NMR (Me_2SO-d_6) δ 2.00 (s, 3 H, CH_3CO), 2.10 (s, 3 H, CH_3CO), 2.15 (s, 3 H, CH_3CO), 4.3–4.5 (m, 3 H, H-4′,5′,5″), 5.50 (t, 1 H, H-2′ or 3′), 5.9 (m, 1 H, H-2′ or 3′), 7.20 (d, 1 H, H-1′, $J_{1',2'}$ = 6.0 Hz), 7.4–7.8 (m, 4 H,

H-4,5,6,7); MS, m/z (relative intensity) 425 ($M^{\bullet+}$, 56), 259 (87), 199 (35), 168 (52), 167 (37), 166 (21), 157 (71), 139 (95), 97 (100).

3-α-D-Arabinofuranosylbenzothiazole-2-thione (15). After the general deblocking procedure was performed on 0.3 g (0.71 mmol) of **13**, the crude material obtained was precipitated from methanol–water to give 0.17 g (80%) of pure **15**: mp 130 °C; UV (MeOH) λ_{max} 325 nm (20.3), 241 (12.0), 227 (12.9); ^{1}H NMR (Me_2SO-d_6) δ 3.3–4.5 (m, 5 H, H-2′,3′,4′,5′,5″), 5.2 (br s, 3 H, OH-2′,3′,5′), 6.30 (d, 1 H, H-1′, $J_{1',2'}$ = 10 Hz), 7.5–8.1 (m, 4 H, H-4,5,6,7); MS, m/z (relative intensity) 299 ($M^{\bullet+}$, 20) 196 (16), 168 (82), 167 (100), 166 (20), 151 (31), 149 (36), 148 (29), 135 (25), 133 (25), 132 (20), 115 (16), 97 (20).

BIOLOGICAL METHODS

Fusarium strains, growth media, and experimental protocols were the same as previously reported (Bounaga, 1980).

The test compounds were first dissolved in ethanol and then added to the growth medium in such a way as to get final concentrations of 0.5, 1.0, and 1.5 mM.

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LITERATURE CITED

Barascut, J. L.; Kam, B. L.; Imbach, J. L. *Bull. Soc. Chim. Fr.* **1976**, 1983.
Bounaga, N. *Can. J. Microbiol.* **1976**, *22*, 636.
Bounaga, N. *Phytopath. Z.* **1980**, *98*, 210.
Durette, D. L.; Horton, D. *J. Org. Chem.* **1971**, *36*, 2669.
Gosselin, G.; Loukil, H. F.; Mathieu, A.; Mesli, A.; Imbach, J. L. *J. Heterocyclic Chem.* **1978**, *15*, 657.
Gosselin, G.; Imbach, J. L.; Townsend, L. B.; Panzica, R. P. *J. Heterocyclic Chem.* **1979**, *16*, 1185.
Guthrie, R. D.; Smith, S. C. *Chem. Ind. (London)* **1968**, 547.
Hudson, G. S.; Johnson, J. M. *J. Am. Chem. Soc.* **1915**, *37*, 2748.
Koch, H. P. *J. Chem. Soc.* **1949**, 401.
Kuszmann, J.; Vargha, L. *Rev. Chim. Acad. Rep. Pop. Roum.* **1962**, *7*, 1025.
Lemieux, R. U.; Lown, J. W. *Can. J. Chem.* **1963**, *41*, 889.
Lemieux, R. U.; Stevens, J. D. *Can. J. Chem.* **1965**, *43*, 2059.
Moore, C. G.; Waight, E. S. *J. Chem. Soc.* **1952**, 4237.
Nishimura, T.; Shimizu, B. *Chem. Pharm. Bull.* **1965**, *13*, 803.
Ryley, J. F.; Wilson, R. G.; Gravestock, M. B.; Poyser, J. P. *Adv. Pharmacol. Chemother.* **1981**, *18*, 50.
Stevens, J. D.; Fletcher, H. G. *J. Org. Chem.* **1968**, *33*, 1799.
Togerson, D. C. *Fungicides, An Advanced Treatise*; Academic: New York, 1969; Vol. 2.
Townsend, L. B. In *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W., Tipson, R. S., Eds.; Wiley-Interscience: New York, 1973; Vol. 2, p 267.
Vogel, A. I. *Practical Organic Chemistry Including Qualitative Organic Analysis*, 3rd ed.; Longmans, Green and Co.: London, 1956; p 451.
Wain, R. L.; Carter, G. A. In *Systemic Fungicides*, 2nd ed.; Marsh, R. W., Ed.; Longman Group: London, 1977; p 6.

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Phosphorylating Intermediates in the Peracid Oxidation of Phosphorothionates, Phosphorothiolates, and Phosphorodithioates

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Oxidation of *O,O*-diethyl phenyl phosphorothionate, phosphorothiolate, and phosphorodithioate with excess *m*-chloroperoxybenzoic acid in aprotic solvents yields primarily diethyl phenyl phosphate, diethylphosphoryl benzenesulfonate and diethylphosphoryl benzenethiosulfonate, respectively, but in methanol the major product in each case is diethyl methyl phosphate. The intermediate phosphorylating agents react more readily with methanol and *n*-propyl alcohol than with isopropyl or *tert*-butyl alcohol. ^{31}P NMR spectra for reactions run at −50 to −20 °C show minor signals 27–34 ppm upfield from the starting materials appropriate for the transient three-membered ring phosphoxathiiranes from the phosphorothionate and phosphorodithioate and the phosphorylsulfenate (formed via the phosphorothiolate *S*-oxide) from the phosphorothiolate. The phosphoxathiiranes and the phosphorothiolate *S*-oxide or their rearrangement products formed on biooxidation of related thiophosphorus toxicants are candidate phosphorylating agents, possibly contributing to their activity as acetylcholinesterase inhibitors and to their detoxification.

Bioactivation of thiophosphorus insecticides to potent phosphorylating agents for acetylcholinesterase (AChE) is proposed to involve cytochrome P450 mediated oxidation of phosphorothionates and phosphorodithioates to the corresponding phosphates and phosphorothiolates (Eto, 1974; Neal, 1980) and of some phosphorothiolates to their *S*-oxide derivatives (Wing et al., 1983, 1984). These bioxidation reactions also cleave phosphorus–sulfur bonds to form a variety of detoxification products (Eto, 1974). Peracid oxidation may be a biomimetic model for these activations of phosphorothionates (Bellet and Casida, 1974;

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