Peroxidative Conversion of Hemigossypol to Gossypol. A Revised Structure for Isohemigossypol

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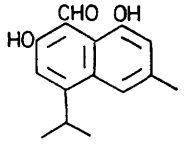
Summary Phytoalexins from *Gossypium* have been identified as hemigossypol (2,3,8-trihydroxy-4-isopropyl-6-methyl-1-naphthaldehyde) and isohemigossypol (2,7,8-trihydroxy-4-isopropyl-6-methyl-1-naphthaldehyde) in separate reports, but comparisons of their u.v.–visible, ^{1}H n.m.r. and mass spectra indicate that they are the same; double resonance studies of the phytoalexin now indicate that the correct structure is hemigossypol, which has been confirmed by dimerization of the phytoalexin to gossypol with the enzyme peroxidase.

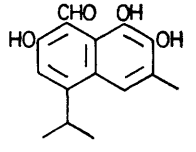
RECENTLY Bell *et al.*[1] reported the isolation and identification of the phytoalexin, hemigossypol (2,3,8-trihydroxy-4-isopropyl-6-methyl-1-naphthaldehyde), (**1**) from *Gossypium* species infected with the fungus *Verticillium dahliae*. Sadykov, *et al.*[2] have also reported the isolation of a phytoalexin from similarly infected *Gossypium*, but they identified it as isohemigossypol, (**2**). Comparisons of the spectra (i.r., ^{1}H n.m.r. and mass) of hemigossypol (**1**)[1,3] and isohemigossypol (**2**)[2] indicate that they are the same compound. Further, when we used the isolation procedures of Sadykov *et al.*, only the phytoalexin previously identified as (**1**) was obtained.

Sadykov, *et al.* selected structure (**2**) for the phytoalexin because of a series of double resonance experiments. Their ^{1}H n.m.r. spectrum ($CDCl_3$) of the compound showed δ 1·42 (6H, d), 2·32 (3H, s br), 3·76 (1H, septet), and 5·55, 6·23, 6·60, 7·46, 11·50 and 14·95 (each 1H, s). Irradiation of the methyl proton (2·32) reportedly increased the intensity of only the aromatic proton at 7·46. The intensities of both aromatic protons (7·46, 6·60) increased equally when the methyl doublet (1·42) of the isopropyl was irradiated. Furthermore, no *meta*-coupling was observed between the aromatic protons.

Because of these results, we analysed the ^{1}H n.m.r. spectrum of hemigossypol (**1**) by double resonance techniques. The proton at C(7) (assigned by comparison with gossypol) appeared as a slightly broadened singlet at 6·65 with a coupling constant of about 0·5 Hz. The proton at C(5) (7·48) appeared as a broad singlet, apparently due to coupling with the C(7), aromatic methyl, and isopropyl



(1)



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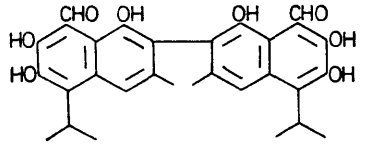

(3)

methine protons. In contrast to the results of Sadykov, *et al.*, when the aromatic methyl protons (2·39) of (**1**) were irradiated, the intensities of both aromatic protons increased with the C(7) proton exhibiting a slightly larger increase than the C(5). This is expected for structure (**1**) because the C(5) proton should also have some coupling with the methine proton of the isopropyl group.

When the methyl protons of the isopropyl group were irradiated we observed no changes in the intensities of the aromatic protons of hemigossypol (**1**), while Sadykov, *et al.*

reported increased intensities of the aromatic protons. Such an increase is difficult to explain with structure (**1**) or (**2**), because the methyl protons of either are too far removed from the aromatic protons to show appreciable coupling.

Irradiation of the proton at 7·48 of hemigossypol (**1**) increased the intensity of the peak at 6·65. Likewise, the intensity of the peak at 7·48 increased when the proton at 6·65 was irradiated. Thus, our double resonance experiments indicate structure (**1**) for the phytoalexin from *Gossypium*.

To confirm structure (**1**) for the phytoalexin, we treated hemigossypol (**1**) with horseradish peroxidase. This enzyme dimerizes aromatic compounds by the intermediate formation of free radicals.[4] Thus, (**1**) but not (**2**), can be converted into gossypol (**3**) by the enzyme.

The major product (**3**) formed by peroxidative dimerization of hemigossypol (**1**) had m.p. 192—196 °C ($CHCl_3$) and mixed m.p. with gossypol of 192—197 °C. The i.r., ^1H n.m.r., u.v.-visible, and mass spectra of the reaction product agreed with those of authentic gossypol (**3**).[3] The structure of gossypol has been estbalished by synthesis.[5] We conclude, therefore, that the structure of the phytoalexin reported by ourselves and Sadykov, *et al.*[2] is hemigossypol (**1**) and not isohemigossypol (**2**).

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