Proposed Structure for a New Phenolic Ketone in Potato Tubers Infected with *Phoma*

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A new tricyclic phenolic ketone (Solanolone) has been isolated from the blue-fluorescent stress zone of potato tubers (cv. Bintje) infected by *Phoma exigua* var. *foveata*. The structure proposed for the compound is 1,2,3,4,4a,10a-hexahydro-6-methyl-3,4,7,10-tetrahydroxy-9(10H)-phenanthrone with the protons at 3, 4, 4a, 10 and 10a all in *trans* configuration.

The biosynthesis of new components and/or the accumulation of low molecular components that normally occur in plants may be part of natural defence systems. Potato tubers are known to produce a blue fluorescent stress zone in the healthy tissue adjacent to infections and wounds. Coumarins and sesquiterpenes in the tissue of this zone in potato tubers infected with the fungus *Phoma exigua* var. *foveata* were the subject of a previous study.[1] A new tricyclic phenolic compound has been isolated from this source and a proposed structure is now reported.

The structural determination was based mainly on ^{1}H and ^{13}C NMR spectra. The molecular weight was 278 according to the mass spectrum determined at 70 and 20 eV. This datum, together with ^{1}H and ^{13}C NMR spectra, led to the molecular formula $C_{15}H_{18}O_5$. The presence of a tetrasubstituted benzene ring with the two protons in the *para* position was indicated in both the ^{1}H and ^{13}C NMR spectra, as were three aliphatic carbons and one aromatic carbon bonded to oxygen. A carbonyl carbon (δ 205.6, s) was also noted in the spectrum. These data and the absence of any other unsaturated carbon in the ^{1}H and ^{13}C NMR spectra suggested a tricyclic structure with one benzene ring.

The ^{1}H and ^{13}C NMR spectra indicated a methyl and a hydroxyl substituent in the benzene ring. The shift of the carbonyl carbon and the IR absorption indicated an aromatic ketone with intramolecular hydrogen bonding.[2] One methine proton appeared as a doublet in ^{1}H NMR at δ 4.51, indicating an α proton to the keto function.[3] Decoupling of this doublet showed that the proton was coupled to a proton in the multiplet at about δ 1.5.

In the ^{13}C NMR spectrum, 2 additional methylene carbons and 5 methine carbons, of which 3 were attached to oxygen, were present. The proton of one of the methine groups appeared as a triplet at δ 3.66 in ^{1}H NMR. Decoupling of this signal did not influence other signals in the spectrum. This indicated that it was a central proton in a 3-carbon chain of 3 methine groups and that the other 2 protons appeared in the multiplet at δ 3.5, which was too close to the decoupling frequency for the decoupling to be noticed. The shifts, however, were in agreement with literature data on similar structural elements.[2,4] The shift value for the proton on the methine group not bonded to oxygen indicated an α position to the benzene ring. Decoupling of the proton appearing at δ 1.5 in ^{1}H NMR spectrum, mentioned above, influenced the multiplet at δ 3.5, indicating that the protons H_{10a} and H_{4a} were vicinal. Decoupling of H_{4a} also slightly influenced the

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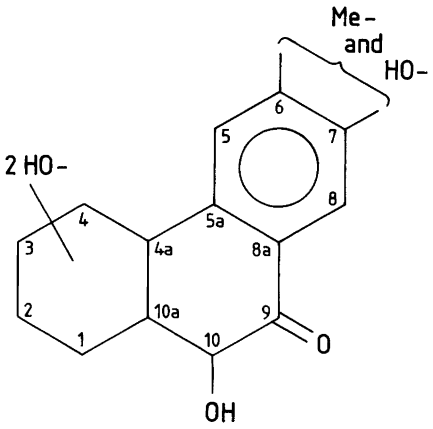

Fig. 1.

aromatic protons. These data suggested the structure shown in Fig. 1.

Because of the small amount of material available, only one chemical transformation was performed to obtain additional information on the structure. After oxidation of the diol function with $NaIO_4$, a small amount of dicarboxylic acid was obtained.[5,6] In the ^1H NMR spectrum, the doublet of the H_{10} (δ 4.49) was decoupled. A weak multiplet at δ 3.1 was influenced but, after decoupling, the signal was still a multiplet. The doublet at δ 4.49 was, of course, influenced by decoupling of the multiplet at δ 3.1. There may also have been a doublet at δ 4.2, originating from H_{4a}, which was influenced, but the low signal-to-noise ratio made this conclusion uncertain. These data and the presence of the 3-carbon chain of methine groups mentioned above suggested the position of the diol function shown in Fig. 2 with the proton at δ 3.1 located adjacent to several other protons in the oxidized product.

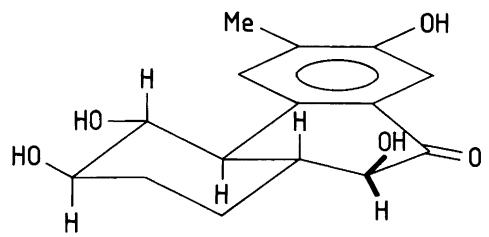

Fig. 2.

The methyl and hydroxy substituents on the benzene ring were located in positions 6 and 7, respectively, or vice versa. The UV spectrum of the compound suggested an *o*- or *m*-hydroxyphenyl ketone moiety rather than *p*-hydroxy substitution.[7] This was further supported by the disagreement with the UV data reported for aromatic diterpenes with a *p*-hydroxyphenyl moiety in the structure, all with the absence of a λ_{max} above 300 nm in the UV spectrum.[8,9] These and NMR data suggested a 7-hydroxy-6-methyl substitution of the benzene ring.

The stereochemistry of the structure was partly established. The coupling constant between the H_{10} and H_{10a} was 12.5 Hz, which clearly showed an a-a orientation of the 2-protons. The coupling constants between the central proton (H_4) in the 3 adjacent methine groups and H_3 and H_{4a} were about 8.5 Hz each. This indicated an all-axial orientation of these 3 protons in a cyclohexane ring.[10] The coupling constant between H_{4a} and H_{10a} could not be established, but the diaxial position of these 2 protons was deduced from the a-a position to H_4 and H_{10}, respectively.

According to the spectral data above, the structure of the compound is suggested to be 1,2,3,4,4a,10a-hexahydro-6-methyl-3,4,7,10-tetrahydroxy-9(10*H*)-phenanthrone (Solanolone) with a stereochemistry according to Fig. 2 or its mirror image.

This compound represents a new type of compound in potato tubers. It was isolated from the blue fluorescent zone in potato tubers infected with *Phoma exigua* var. *foveata*. It has not been investigated as to whether the compound is present in healthy tubers or whether it is produced in the fluorescent zone after the infection, and if so, whether the metabolite is regularly produced following infections and stress.

Experimental

General. The NMR spectra were recorded at 89.6 MHz (^1H NMR) and 22.5 MHz (^{13}C NMR) in $CDCl_3$ solutions, except the oxidized sample, which was recorded in acetone-d_6, with TMS as internal standard. The mass spectra were obtained on a Finnigan 4021 instrument at 70 or 20 eV. The column chromatography was followed by TLC. The plates were inspected in UV light

and sprayed with diazotized sulfanilic acid in 10 % Na_2CO_3.

Isolation. The extraction of 6 kg of the blue fluorescent stress zone of potato tubers (cv. Bintje) infected with *Phoma exigua* var. *foveata* has been described previously.[11] The compound was isolated from the CH_2Cl_2 extract in the extraction. After column chromatography on silica gel, using $CHCl_3$/EtOH with increasing EtOH content (5–50 %) as eluent and rechromatography twice on silica gel with toluene/MeOH (10:3) and $CHCl_3$/EtOH (15:1), 13 mg of pure compound were obtained.

Isolated compound. Amorphous, $[\alpha]_D^{22}$ 51.8° (MeOH, c 1.00). ^{1}H NMR: δ 7.04 (1H, s), 6.72 (1H, br, s), 4.74 (1H, br, OH), 4.51 (1H, d, J 12.5 Hz), 3.66 (1H, t, J ~8.5 Hz), 3.5 (2H, m), 2.34 (3H, s), 1–2.5 (m). ^{13}C NMR: δ 205.6 (s), 162.8 (s), 149.7 (s), 147.0 (s), 117.5 (d), 117.2 (d), 112.9 (s), 75.2 (d), 73.6 (d), 72.6 (d), 52.6 (d), 46.1 (d), 29.6 (t), 26.7 (t), 22.4 (q). MS, 70 eV [m/z (% of rel.int.)]: 278 $[M]^+$ (48), 260 (68), 242 (26), 219 (61), 135 (83), 91 (59), 43 (100). MS, 20 eV [m/z (% of rel. int.)]: 278 $[M]^+$ (68), 260 (100), 242 (33), 219 (73), 135 (33), 91 (7), 43 (25). IR, $\nu_{max}^{CHCL_3}$ cm^{-1}: 1620, 1615, 1560. UV, λ_{max}^{MeOH} nm: 218 (sh), 267, 335.

Oxidation of the isolated compound. 10 mg of the compound dissolved in 3 ml of H_2O/HOAc (2:1) were mixed with 1 ml of 0.04 M $NaIO_4$ and allowed to react overnight in the dark.[5] The dialdehyde produced was oxidized to dicarboxylic acid by addition of 5 mg sulfamic acid and a solution of 5.6 mg $NaClO_2$ in 2 ml of H_2O.[6] After 0.5 h, the solution was extracted with EtOAc, dried and evaporated. The reaction mixture was chromatographed on silica gel 60 with toluene/MeOH (10:3) as eluent. ^{1}H NMR (acetone-d_6, 50 °C): δ 7.11 (s), 6.67 (s), 4.49 (d), 3.15 (m).

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