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STRUCTURE OF RISHITINONE, A STRESS METABOLITE FROM DISEASED POTATO TUBERS[1)]

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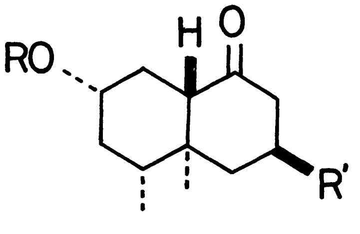
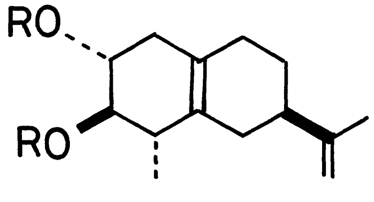
Structure and configuration of rishitinone, a stress metabolite isolated from diseased potato tubers, was determined to be formula **1** on the basis of the chemical and spectral evidence.

In a continuing study on phytoalexins produced by tuber tissues of potatoes (Solanum tuberosum × S. demissum) infected by an incompatible race of Phytophthora infestans,[2)] we isolated a new sesquiterpene, designated as rishitinone (**1**), from the chloroform extracts of the tissues, which was not detected in the corresponding extracts of healthy tissues and showed a comparable fungitoxicity with that of rishitin[2)] (**2**). We describe herein the isolation and structure elucidation of the stress metabolite (**1**).

Neutral chloroform extracts (80 g), obtained from the diseased potato tuber tissues (300 kg), were separated as described previously[2)] to give "rishitin-rich fraction" (Fraction F,[2)] 5.5 g), which on acetylation followed by chromatogarphy afforded a semi-crystalline mixture of acetates. Fractional recrystallization of the mixture gave pure rishitin diacetate[2)] (**2a**, 3.3 g), mp 65-67 °C, and crude oily rishitinone acetate (**1a**, 0.11 g), which was submitted to purification by chromatography to yield rishitinone acetate (**1a**), oily and $[\alpha]_D$ -2.4°,[3)] in pure state. The acetate (**1a**) was then hydrolyzed (K_2CO_3 in CH_3OH, room temp, 1 h) to give rishitinone (**1**), mp 72-75 °C and $[\alpha]_D$ +10.1°.



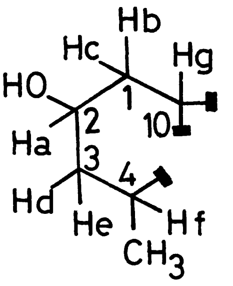
1 R=H, R'=$C(CH_3)=CH_2$
1a R=Ac, R'=$C(CH_3)=CH_2$
3 R=H, R'=$CH(CH_3)_2$



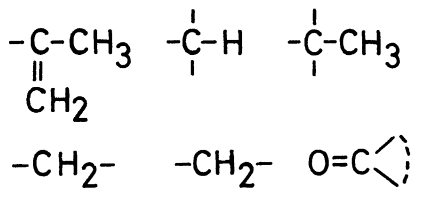
2 R=H
2a R=Ac

Rishitinone (**1**) was analyzed for $C_{15}H_{24}O_2$ (m/e 236, M^+) and converted by hydrogenation (Pd-C in C_2H_5OH) into dihydrorishitinone (**3**), mp 80-82 °C and $[\alpha]_D$ +7.2°. The IR and ^1H-NMR spectra[3)] indicated the presence of the following structural units: a t-methyl group [**1**, δ 0.76 (3H, s)]; a s-methyl [**1**, δ 0.84 (3H, d, J = 7 Hz)]: an isopropenyl [**1**, ν_{max} 1615 and 887 cm^{-1}; δ 1.76 (3H, s) and 4.77 (2H, br s); **3**, ν_{max} 1388 and 1372 cm^{-1}, δ 0.91 (6H, d, J = 6 Hz)], a carbonyl [**1**, ν_{max} 1710 cm^{-1}]; a <u>sec</u>-hydroxyl [**1**, ν_{max} 3500 cm^{-1}; δ 3.65 (1H, m, W_H = 25 Hz); **1a**, ν_{max} 1739 and 1248 cm^{-1}; δ 2.02 (3H, s) and 4.77 (1H, m, W_H = 25 Hz)]. The ^{13}C-NMR spectra obtained under proton-noise decoupled and single-frequency off-resonance decoupled conditions [δ 12.9, 15.5, and 20.4 (each $-CH_3$); 30.2, 39.3, 42.8, and 46.0 (each $-CH_2-$); 41.3, 41.5, 56.1, and 69.5 (each $-\overset{|}{C}H-$); 40.7 (■)$^{3)}$; 109.9 ($=CH_2$); 147.4 ($=\overset{|}{C}-$); 211.1 ($-\overset{|}{C}=O$)] provided additional information of carbon atoms, indicating that the ketone (**1**) possesses a bicyclic skeleton.

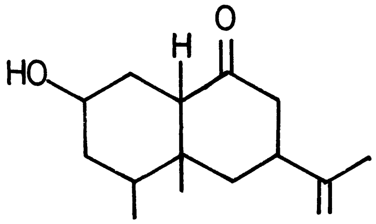
The ^1H-NMR spectra of **1** in the presence of the shift reagent $Eu(dpm)_3$ (0.2 and 0.4 mol equiv) effected downfield shifts of all the signals, and exhibited one-proton broad signals (W_H = 25 Hz) at δ 6.80 and 10.50 in the respective spectra, which were reasonably assigned to the proton on the carbon bearing the hydroxyl group. These spectra, coupled with spin-decoupling studies (Table 1), strongly suggested that the ketone (**1**) would probably involve a partial formula A. In view of the remaining nine carbon units (formula B) deduced from the afore-mentioned



A



B



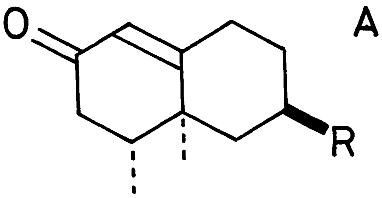
C

Table 1. The ^1H-NMR spectra of rishitinone (**1**) in the presence of the shift reagent $Eu(dpm)_3$ (CCl_4, 100 MHz) and results of the spin-decoupling studies

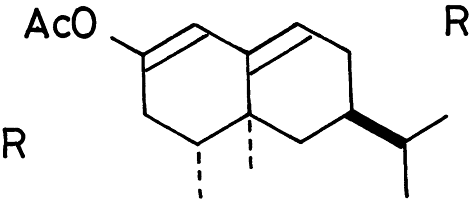
Protons irradiated (δ and Hz)	Observed multiplicity change (δ and Hz)
Mole ratio of **1**:$Eu(dpm)_3$ 5:1	
6.80 [Ha (C-2), br (W_H = 25)]	4.00 [Hb, dt → dd (J = 12 and 4)]
	3.20 ∿ 3.80 [Hc ∿ He, br m → changed]
4.00 [Hb (C-1), dt (J = 12 and 4)]	6.80 [Ha, br → changed]
3.50 [Hc ∿ He (C-1 ∿ C-3), br m]	6.80 [Ha, br → changed]
	2.30 [Hf, br → changed]
2.34 [Hf (C-4), br]	1.23 [CH_3, d → s]
1.23 [CH_3 (C-4), d (J = 7)]	2.30 [Hf, br → dd (J = 11 and 3)]
Mole ratio of **1**:$Eu(dpm)_3$ 5:2	
10.5 [Ha (C-2), br (W_H = 25)]	5.6 ∿ 6.4 [Hb ∿ He, br m → changed]
6.12 [Hb and Hc (C-1), br m]	4.12 [Hg, dd → s]
4.12 [Hg (C-10), dd (J = 12 and 4)]	6.0 ∿ 6.3 [Hb ∿ Hc, br m → changed]

spectra, specially from the ^{13}C-NMR spectra, formula C is presented as one of the most plausible planar structures for **1**. This structure was confirmed by correlation between reshitinone (**1**) and (+)-nootkatone[4)] (**4**).

(+)-Nootkatone (**4**) was submitted to partial hydrogenation in benzene containing tris(triphenylphosphine)chlororhodium (room temp, 14 h) to give dihydronootkatone (**5**), oily and $[\alpha]_D$ +167°, in an 81% yield; ν_{max} 1670 cm^{-1}; δ 0.69, 0.72, and 0.78 (each 3H, d, J = 7 Hz, 12-, 13-, and 14-H), 1.08 (3H, s, 15-H), and 5.78 (1H, s, 1-H). The compound (**5**) was converted by a modification of the Edward procedure[5)] into dienol acetate (**6**), oily and $[\alpha]_D$ +45.3°, in a 69% yield; m/e 262 (M^+); ν_{max} 1759 and 1664 cm^{-1}; δ 2.12 (3H, s, $OCOCH_3$), 5.42 (1H, m, W_H = 10 Hz, 9-H), and 5.71 (1H, s, 1-H), which on hydride reduction ($NaBH_4$) produced a 1:5 mixture of homoallylic alcohols (**7**) and (**8**). The major product (**8**) was assigned an equatorial alcohol on the basis of the following spectral data: **7**, oily and $[\alpha]_D$ -30.7°; m/e 222 (M^+); ν_{max} 3300, 1382, and 1365 cm^{-1}; δ 3.99 (1H, m, W_H = 8 Hz, 2-H) and 5.43 (1H, m, W_H = 12 Hz, 9-H): **8**, oily and $[\alpha]_D$ -34.2°; m/e 222 (M^+); ν_{max} 3320, 1381, and 1368 cm^{-1}; δ 3.56 (1H, m, W_H = 25 Hz, 2-H) and 5.38 (1H, m, W_H = 12 Hz, 9-H). The hydroxyl group of the latter (**8**) was then protected with <u>t</u>-butyldimethylsilyl (TBDMS) chloride and imidazole in dimethylformamide. The resulting silyl ether (**8a**), oily and $[\alpha]_D$ -16.3°; m/e 337 (M^+ + 1); ν_{max} 1100 and 1258 cm^{-1}, obtained in a 76% yield, underwent hydroboration followed by oxidation with alkaline hydrogen peroxide, producing a mixture of two isomeric alcohols (**9**) and (**10**) and the starting alkene (**8a**), which were separated by chromatography over silica gel in 23 (crude), 42 (pure), and 8% yields, respectively. The major product (**10**) was assigned 9β-hydroxy-<u>trans</u>-valencane structure on the basis of the spectral data and the steric manner (<u>cis</u>-addition from the less-hindered side) of this reaction:[6)] **10**, oily and $[\alpha]_D$ +38.0°; m/e 354 (M^+); ν_{max} 3360, 1258, and 1100 cm^{-1}; δ 3.49 (1H, dt, J = 4.5 and 10 Hz, 9-axial-H) and 3.58 (1H, m, W_H = 25 Hz, 2-H); acetate (**10a**) of **10**, oily and $[\alpha]_D$ +35.5°; δ 3.76 (1H, m, W_H = 25 Hz, 2-H) and 4.74 (1H, dt, J = 4 and 11 Hz, 9-H). The <u>trans</u>-decalol (**10**) was then oxidized with Sarett reagent (room temp, 15 min) to yield the corresponding ketone (**11**) in a quantitative yield, mp 51-53 °C and $[\alpha]_D$ +2.1°; m/e 352 (M^+); ν_{max} 1717 cm^{-1}; δ 0.66 (3H, s, 15-H) and 0.90 (9H, br m, 12-, 13-, and 14-H), which was hydrolyzed without epimerization at C-10 in a 3:1:1 mixture of acetic acid, water, and tetrahydrofuran to give dihydrorishitinone (**3**) in a 95% yield, identical with an authentic specimen derived from natural (+)-rishitinone in all respects. Supporting the assigned configuration, the ORD spectrum[3)] of the ketone (**3**) showed a typical positive Cotton effect, whose amplitude (a = +26.6°) was in good accord with that (a = +26°) reported for a known ketone[7)] (**12**), which had been derived from natural (-)-aristolochene, one of valencenes. All these results indicate the structure and configuration of (+)-rishitinone to be represented correctly by formula **1**.



4 R=$C(CH_3)=CH_2$
5 R=$CH(CH_3)_2$



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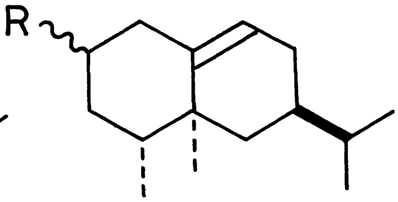
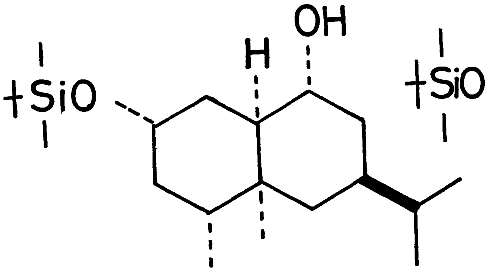


7 R=β-OH
8 R=α-OH
8a R=α-OTBDMS



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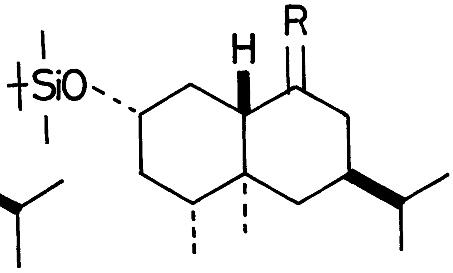
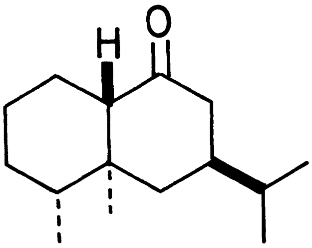


10 R=β-OH, α-H
11 R=O



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References and Notes

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3) a) The optical rotations were obtained in ethanol. The IR and NMR spectra were measured in liquid state (oil) or in Nujol (solid), and in chloroform-d, respectively, unless otherwise stated. b) The abbreviation ▬ denotes a carbon atom bearing no hydrogen atom(s).

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