Structure of Rishitin M-1 and M-2, Metabolites of Rishitin in Healthy Potato Tuber Tissues

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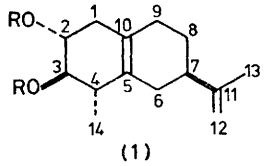
Summary The title compounds metabolites of rishitin (**1a**) in healthy potato tuber tissues, are determined to possess structures (**2a**) and (**3a**).

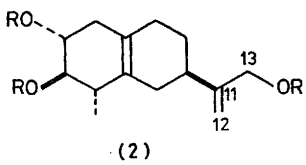
RISHITIN (**1a**), an antifungal norsesquiterpene produced in diseased potato tuber tissues and classified as a phytoalexin,[1] has recently been reported to be metabolized, to a significant extent, when it is incubated exogenously with healthy tissues of white potato (Rishiri, *Solanum tuberlosum* × *S. demissum*).[2] We describe herein the isolation and structure elucidation of two metabolites (**2a**) and (**3a**).

Methanol extracts of thin slices of aged potato, treated with rishitin (**1a**) in water–acetone (9 : 1) at 23—24 °C for 24 h, were concentrated and extracted with chloroform. The chloroform extracts† were fractionated sequentially by repeated column and thin-layer chromatography, resulting in the isolation of two norsesquiterpenes (**2a**) and (**3a**) in 14 and 1·8% yields [from (**1a**)], respectively. These compounds (**2a**) and (**3a**), designated as rishitin M-1 and M-2, could not be detected in the chloroform extracts from slices not incubated with (**1a**) and were indistinguishable



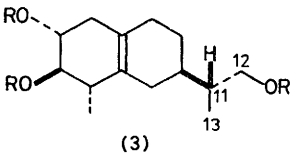
(1)

a, R = H
b, R = COMe



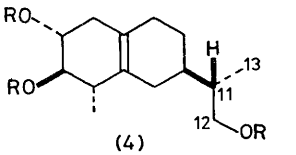
(2)

a; R = H
b; R = COMe
c; R = $COC_6H_3(NO_2)_2$-3,5



(3)

a, R = H
b, R = COMe
c, R = $COC_6H_3(NO_2)_2$-3,5



(4)

a; R = H
b; R = COMe
c; R = $COC_6H_3(NO_2)_2$-3,5

† Chloroform-insoluble aqueous extracts also contain several components, which are regarded as metabolites of (**1a**) and which are more hydrophilic than (**2a**) and (**3a**).

from the corresponding ^{14}C labelled samples in the chloroform extracts from slices treated with [^{14}C]rishitin in the same manner on two-dimensional thin-layer chromatograms.

Rishitin M-1 (**2a**), oil, $C_{14}H_{22}O_3$ (*m/e* 238, M^+), $[\alpha]_D$ −33·2° (EtOH), gave the triacetate (**2b**), oil, $C_{20}H_{28}O_6$ [*m/e* 304 (M^+ − AcOH)], $[\alpha]_D$ −17·1° (EtOH), and the tris-(3,5-dinitrobenzoate) (**2c**), m.p. 220·5—222·5 °C, $[\alpha]_D$ −44·8° ($CHCl_3$), which were reconverted by saponification into (**2a**). The i.r. and ^1H and ^{13}C n.m.r. spectra of (**2a**) and (**2b**) revealed that (**2a**) differed from (**1a**) only in the isopropenyl unit: (**2a**) δ ($CDCl_3$) (^1H) 4·10 (2H, s, 13-CH_2-OH),[3] and 4·81 and 5·05 (each 1H, s, 12-CH_2):[3] (**2a**) δ ($CDCl_3$) (^1H) 2·01, 2·05, and 2·08 (each 3H, s, 3OCO*Me*), 4·57 (2H, s, 13-CH_2OAc), and 4·90 and 5·08 (each 1H, s, 12-CH_2); δ ($CDCl_3$) (^{13}C), 31·5, 36·3, 26·4, 147·0, 112·0, and 66·2 (6-, 7-, 8-, 11-, 12-, and 13-C, respectively); *cf.*, rishitin diacetate (**1b**), 31·4, 40·4, 26·6, 148·9, 109·3, and 21·1. These data indicate that rishitin M-1 (**2a**) is 13-hydroxyrishitin.

Rishitin M-2 (**3a**), oil, $C_{14}H_{24}O_3$ [*m/e* 222 (M^+ − H_2O)], $[\alpha]_D$ −17·7° (EtOH), also gave a triacetate (**3b**), oil, $C_{20}H_{30}O_6$ [*m/e* 306 (M^+ − AcOH)], $[\alpha]_D$ −11·5° (EtOH) and −8·3° ($CHCl_3$), and a tris(3,5-dinitrobenzoate) (**3c**), m.p. 198—200 °C, $[\alpha]_D$ −27·2° ($CHCl_3$), which were also reconverted by saponification into (**3a**). The i.r. and ^1H and ^{13}C n.m.r. spectra indicated that (**3a**) was formed by hydration of (**1a**) at the isopropenyl double bond [–(Me)C=CH_2 → –(Me)CHCH_2OH]: (**3a**) δ ($CDCl_3$ + D_2O) (^1H) 0·93 (3H, d, *J* 6 Hz, 13-Me) and 3·58 (3H, br m, 12-CH_2OH and 2-C*H*OH): (**3b**) δ ($CDCl_3$) (^1H) 0·94 (3H, d, *J* 6·5 Hz, 13-Me), 2·02 and 2·07 (3H and 6H, each s, 3OCO*Me*), 3·93 and 4·08 (each 1H, double ABq, *J* 11, 6 and 11, 5·5 Hz, 12-CH_2OAc); δ ($CDCl_3$) (^{13}C) 30·4, 21·1, 24·6, 35·4, 68·0, and 14·2 (6-, 7-, 8-, 11-, 12-, and 13-C, respectively). These spectra were superimposable with the corresponding spectra of authentic samples of (11*S*)-12-hydroxy-11,12-dihydrorishitin[4] (**4a**), $[\alpha]_D$ −8·7° (EtOH), its triacetate[4] (**4b**), $[\alpha]_D$ −0·2° (EtOH) and +4·6° ($CHCl_3$),‡ and its tris(3,5-dinitrobenzoate) (**4c**), m.p. 200—202 °C, $[\alpha]_D$ −33·5°, which had been prepared from (−)-α-santonin in an unambiguous manner.[4] However, in view of the slight but definite difference between the optical rotations of (**3a**) and (**4a**) and their respective derivatives, rishitin M-2 is represented most satisfactorily by structure (**3a**), an (11*R*)-epimer of (**4a**). It is to be noted that both rishitin M-1 (**2a**) and M-2 (**3a**) are not only ineffective against the growth of the pathogen at 2·5 × 10^{-3} M but also non-toxic against the healthy tissues at 1 × 10^{-2} M in water.§

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‡ The optical rotation, $[\alpha]_D$ +1·7°, of compound (**27a**) in ref. **4** should be revised to +4·6°.

§ Rishitin (**1a**) is toxic against the healthy tissues at 1 × 10^{-3} M concentration in water.

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