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TRANSFORMATIONS OF STEROIDS AND THE STEROIDAL ALKALOID, SOLANINE, BY *PHYTOPHTHORA INFESTANS*

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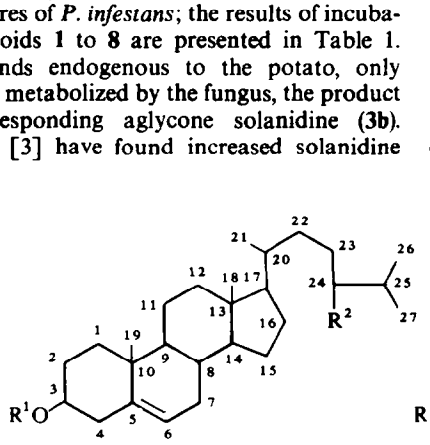
Key Word Index—*Phytophthora infestans*; fungus; solanine; steroids; transformations; metabolism.

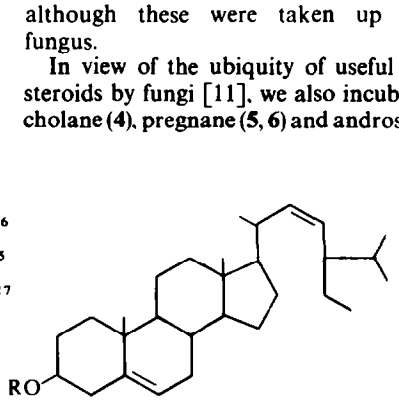
Abstract—The following steroids and steroidal alkaloids have been incubated with the blight fungus *Phytophthora infestans*: androst-4-ene-3,17-dione, cholesterol, cholesteryl acetate, cholesteryl myristate, cholesteryl palmitate, cholesteryl stearate, dehydroisoandrosterone, 6α-hydroxy-androst-4-ene-3,17-dione, 6β-hydroxyandrost-4-ene-3,17-dione, 11α-hydroxyprogesterone, pregnenolone, progesterone, sitosterol, sitosteryl acetate, solanidine, solanine, stigmasterol, stigmasteryl acetate and testosterone. No hydroxylation was observed, but the fungus is able to oxidize alcohol functions at C-3β, C-6α, C-11α and C-17β to carbonyl. In addition, hydrolysis of acetate to hydroxyl at C-3β, and of solanine to solanidine, was observed. The relationship between metabolism and the nature of substitution at C-17β is discussed.

The recent interest in the metabolites produced by diseased potato tubers (the 'stress metabolites') [1–4] and the reported changes in the levels and distribution of secondary metabolites of the potato upon infection by the blight fungus *Phytophthora infestans* [5, 6], prompted us to undertake a systematic study of the fate of steroids (both those endogenous to the potato and others) and the steroidal potato alkaloid, solanine, when incubated exogenously with *P. infestans*. The suggestion that the occurrence of fetal central nervous system defects such as anencephaly may be linked to consumption of blight infected potatoes by the mother during pregnancy, and thus to a specific phytoalexin [7], was also of concern to us in undertaking this study.

Incubations were performed with actively growing and resting cultures of *P. infestans*; the results of incubations of the steroids **1** to **8** are presented in Table 1. Of the compounds endogenous to the potato, only solanine (**3a**) was metabolized by the fungus, the product being the corresponding aglycone solanidine (**3b**). Zacharius *et al.* [3] have found increased solanidine levels in potato tubers infected with *P. infestans*, and have suggested that this was caused by the activity of hydrolytic potato enzymes on **3a**; our work indicates that the fungus itself can convert solanine (**3a**) into solanidine (**3b**). The endogenous steroids cholesterol (**1a**), sitosterol (**1f**) and stigmasterol (**2a**) were not metabolized by *P. infestans*, although active uptake of these steroids by the fungus was observed, recovered steroid being principally in the mycelial extract. This is consistent with the heterothallic nature of *P. infestans*, which relies on an external source of steroids for sexual reproduction [8, 9], and is stimulated to increased vegetative growth by the presence of steroids [10]. The acetates **1b**, **1g** and **2b** were partially hydrolysed to the corresponding sterols by *P. infestans*, but no hydrolysis of the long chain esters **1c** to **1e** was observed, although these were taken up efficiently by the fungus.

In view of the ubiquity of useful transformations of steroids by fungi [11], we also incubated steroids of the cholane (**4**), pregnane (**5**, **6**) and androstane (**7**, **8**) skeletons





1a $R^1 = R^2 = H$
1b $R^1 = Ac$; $R^2 = H$
1c $R^1 = CO(CH_2)_{12}CH_3$; $R^2 = H$
1d $R^1 = CO(CH_2)_{14}CH_3$; $R^2 = H$
1e $R^1 = CO(CH_2)_{16}CH_3$; $R^2 = H$
1f $R^1 = H$; $R^2 = C_2H_5$
1g $R^1 = Ac$; $R^2 = C_2H_5$

2a R = H
2b R = Ac

Table 1. Steroid transformations by *Phytophthora infestans*

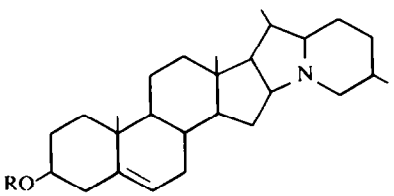
Substrate	Amount (mg)	Incubation period (hr)	Conditions	*P. infestans*	Extract (mg)*	Products (%)
1a	50–900	16–528	direct	NB, ATCC	10–150	**1a** (10) **1a** (70)
1a	150	24	autoclaved	NB	99 25	**1a** (57) **1a** (10)
1b	400	48	direct	NB	200 160	**1b** (40), **1a** (5) **1b** (40)
1c–1e, 2a	400	24	direct	NB, ATCC	0–25 200–370	— **1c–1e, 2a** (50–95)
2b	75	48	direct	NB	30 40	**2b** (25), **2a** (5) **2b** (30), **2a** (10)
1f	30	96	direct	NB	5 35	**1f** (10) **1f** (80)
1g	75	72	direct	NB	40 30	**1g** (50) **1g** (15), **1f** (5)
3a	180	96	direct	ATCC	— 3	**3b** (trace)
3a	300	72	direct	NB	5 15	**3b** (2) —
3b.HCl	50	48	replacement	NB	25 10	**3b** (40) **3b** (20)
4	75	96	direct	NB	25 45	**4** (30) **4** (40)
5a, 7a	75	48	direct	NB	20 45	**5a** (20), **7a** (45) **5a** (70), **7a** (45)
5b	525	96	direct	NB	140 250	**5b** (35), **5c** (trace) **5b** (50)
6	375	72	direct	NB	80 170	**5a** (20) **6** (40), **5a** (trace)
7b	150	72	direct	NB	60 40	**7b** (30), **7a** (trace) **7b** (25)
7c	100	24	direct	NB	110 140	**7c** (25), **7e** (10) **7c** (35)
7d	100	24	direct	NB	100 150	**7d** (40) **7d** (35)
8	750	96	direct	NB	230 480	**8** (25), **7a** (trace) **8** (60)

* Weights are given for the extracts of medium (top line) and mycelia (bottom line).

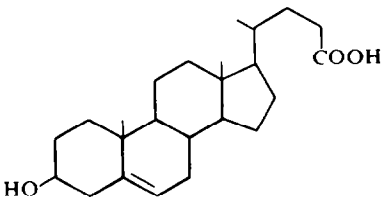
with *P. infestans*. There appeared to be little, if any, preferential uptake of these steroids by *P. infestans*. Alcohol functions at C-11α (of **5b**), C-17β (of **7b**) and C-6α (of **7c**) were oxidized to carbonyl (giving **5c**, **7a** and **7e**, respectively) in low yield, while the C-3β alcohol of **6** and **8** was oxidized to give, with concomitant rearrangement of the Δ^5 bond, the corresponding Δ^4-3-ketones **5a** and **7a**, respectively. No metabolism of the cholane **4** or the C-6β alcohol **7d** was observed.

The steroids oxidized by *P. infestans* (pregnanes and androstanes) are among those listed as inactive in promoting sexual reproduction in *Phytophthora* species [12], while those active (cholestanes and stigmastanes, **1** and **2**) are not metabolized by oxidation. It thus appears that although *P. infestans* is capable of metabolism of steroids by both oxidation and hydrolysis (of acetate esters), the cholestanes and stigmastanes are preferentially utilized by the fungus in promoting sexual reproduction and vegetative growth.

By direct incubation of compounds endogenous in



3a R = glycoside
3b R = H



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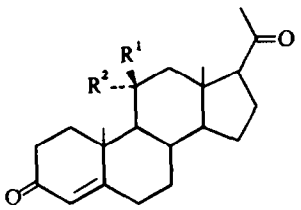
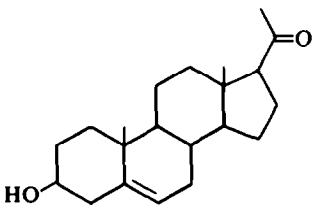


5a $R^1 = R^2 = H$
5b $R^1 = H; R^2 = OH$
5c $R^1 + R^2 = O$



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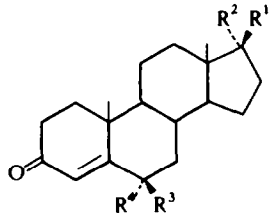
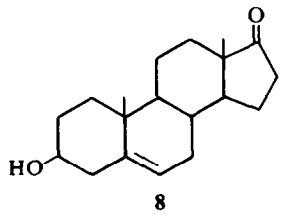


7a $R^1 + R^2 = O; R^3 = R^4 = H$
7b $R^1 = OH; R^2 = R^3 = R^4 = H$
7c $R^1 + R^2 = O; R^3 = H; R^4 = OH$
7d $R^1 + R^2 = O; R^3 = OH, R^4 = H$
7e $R^1 + R^2 = R^3 + R^4 = O$



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the potato with *P. infestans*, we have therefore shown that changes in solanine and solanidine levels upon infection [5, 6] may be attributed directly to the hydrolytic activity of the fungus, and that fungal metabolism of the potato steroids, although a possibility, is less likely than direct utilization for reproduction and growth.

EXPERIMENTAL

Apparatus, materials and methods. Mps are uncorr. ^1H NMR spectra were measured in $CDCl_3$ or DMSO-d_6 and with TMS as internal standard. Column chromatography was performed on Si gel or florisil, TLC on Merck Si gel 60F-254 (0.25 mm), and PLC on Merck Si gel F-254 (2.0 mm). Plates were developed in 4% MeOH in Et_2O and spots visualized by spraying with Allen's reagent.

Phytophthora infestans, obtained both from the American Type Culture Collection (ATCC 16981), and the Agriculture Canada Research Station, Fredericton, New Brunswick (NB), was maintained on slopes of lima bean agar (Difco); transfers were made at intervals of not more than four weeks. No qualitative differences were observed in parallel incubations with fungus from both sources.

Substrates. With the exception of those listed below, all the steroids used in this study were commercial samples which were utilized without further purification.

Cholesterol (**1a**) was purified as described by Fieser [13] and stored under N_2 at $-20°$. Sitosterol (**1f**) and sitosteryl acetate (**1g**) were prepared from stigmasterol (Sigma) by the procedure of ref. [14]. Solanine was isolated from potato sprouts by the published procedure [15] and solanidine prepared from it by acid hydrolysis of the sugar moieties [16].

Incubations with P. infestans. *P. infestans* was grown on a liquid medium prepared as follows. Finely diced potatoes (300 g) were boiled in 500 ml tap H_2O for 1 hr. After passing through cheesecloth, the filtrate was made up to 1 l. with H_2O and the following added: glucose (20 g), peptone (10 g), $FeCl_3$ (0.02 g) and calcium nitrate (0.57 g). The medium was placed in 1 l. flasks (150 ml per flask) and then sterilized at 20 psi for 20 min. Each flask was inoculated by transferring a slice of agar containing fungal mycelia from a growing slope. The cultures were then grown on a New Brunswick gyrotory shaker (140 rpm) at 20–22°. Subsequent inoculation was achieved by transferring growing mycelia via a glass tube from a growing culture (in liquid medium) to fresh medium.

After the culture had grown sufficiently (typically 5–7 days from transfer inoculation), substrate was added, using either direct addition or replacement culture techniques. In the latter, the growing fungus was filtered off, rinsed with distilled H_2O and re-introduced into a 1 l. flask containing 150 ml distilled H_2O; substrate was then added to the flask. For solanine incubations, 30 mg solanine in 1 ml DMSO was added via syringe to each flask. Solanidine hydrochloride (30 mg) was slurried in distilled H_2O and added via syringe. For the other steroids employed, 75 mg in 1–1.5 ml of DMF was added to each flask.

Following a suitable incubation period (see Table 1) the contents of the flask were filtered; the filtrate was extracted × 3 with CH_2Cl_2, and the mycelium macerated with CH_2Cl_2 in a blender. Each extract was then dried and evapd, prior to analysis by TLC and column chromatography.

The results of the incubations of **1** to **8** are presented in Table 1. Products were isolated by preparative layer or column chromatography, and were identified by comparison of physical and spectral data with those of authentic samples.

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