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SHORT COMMUNICATION

THE OXIDATION OF DESOXOROSENONOLACTONE BY *TRICHOTHECIUM ROSEUM*

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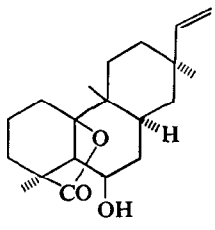
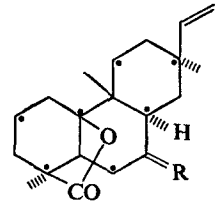
Abstract—Isotopic incorporation and production/time studies confirm that desoxorosenonolactone is oxidized to rosenonolactone and rosololactone by cultures of *Trichothecium roseum* Link.

INTRODUCTION

THE FUNGUS *Trichothecium roseum* Link produces the related diterpenoid metabolites rosololactone[1–5] (I), rosenonolactone[1,2,4–6] (II), desoxorosenonolactone[4] (III), 6β-hydroxy-rosenonolactone,[7] isorosenolic acid[8] and rosein III.[2,9] Detailed studies have been made of



Rosololactone (I)



Rosenonolactone (II; R = O)
Desoxorosenonolactone (III; R = H_2)

[1] A. ROBERTSON, W. R. SMITHIES and E. TITTENSOR, *J. Chem. Soc.* 879 (1949).
[2] G. G. FREEMAN, R. I. MORRISON and S. E. MICHAEL, *Biochem. J.* **45**, 191 (1949).
[3] A. HARRIS, A. ROBERTSON and W. B. WHALLEY, *J. Chem. Soc.* 1807 (1958); M. R. COX, G. A. ELLESTAD, A. J. HANNAFORD, I. R. WALLWORK, W. B. WHALLEY and B. SJOBERG, *J. Chem. Soc.* 7257 (1965).
[4] W. B. WHALLEY, B. GREEN, D. ARIGONI, J. J. BRITT and C. DJERASSI, *J. Am. Chem. Soc.* **81**, 5520 (1959).
[5] A. I. SCOTT, S. A. SUTHERLAND, D. W. YOUNG, L. GUGLIELMETTI, D. ARIGONI and G. A. SIM, *Proc. Chem Soc.* 19 (1964); C. DJERASSI, B. GREEN, W. B. WHALLEY and C. G. DE GRAZIA, *J. Chem. Soc.* 624 (1966).
[6] A. HARRIS, A. ROBERTSON and W. B. WHALLEY, *J. Chem. Soc.* 1799 (1958); B. GREEN, A. HARRIS, W. B. WHALLEY and H. SMITH, *Chem. Ind.* 1369 (1958); G. A. ELLESTAD, B. GREEN, A. HARRIS, W. B. WHALLEY and H. SMITH, *J. Chem. Soc.* 7246 (1965).
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[8] A. I. SCOTT, D. W. YOUNG, S. A. HUTCHINSON and N. S. BHACCA, *Tetrahedron Letters* 849 (1964).
[9] A. R. JONES, Ph.D. Thesis, University of Manchester (1966).

the conversion of acetic[10] and mevalonic[10–12] acids and higher alcohols[12,13] into rosenono- and rosolo-lactones, and recently[12,13] evidence has been presented that the concluding biosynthetic steps in the formation of these two diterpenes involve oxidation of desoxorosenonolactone. We provide here independent evidence that desoxorosenonolactone (III) is a specific precursor of both rosenonolactone (II) and rosololactone (I) in *T. roseum*, a conversion in accord with the known ability[14] of this organism to hydroxylate the steroid nucleus.

RESULTS

^{14}C-Desoxorosenonolactone (III) was prepared by Wolff-Kishner reduction of the hydrazone of [1-^{14}C]acetate-derived rosenonolactone* and therefore carries the known[10]

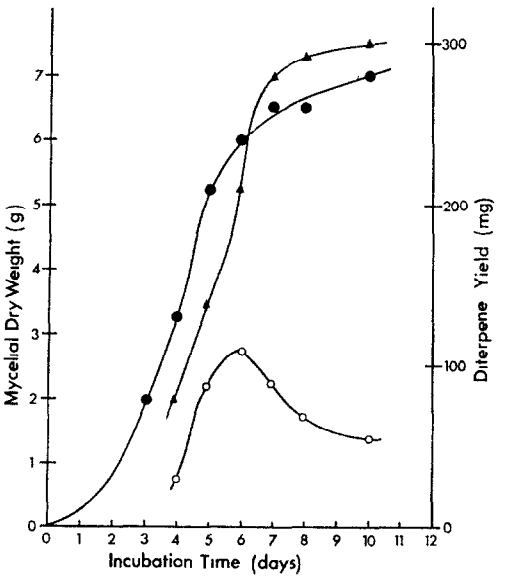

FIG. 1. METABOLISM OF *T. roseum*. RESULTS REPRESENT THE AVERAGE OF TWO RUNS, EACH USING THREE FLASKS OF SHAKEN CULTURE.

● Mycelium
○ Desoxorosenonolactone
▲ Rosenono- and rosolo-lactone combined

isotope pattern (indicated by •). That the "natural" stereochemistry adjacent to the original ketone has been retained without epimerization during this preparation follows from comparison of the physical properties of the product with those of natural desoxorosenonolactone.[4] This material was incorporated by shaken cultures of *Trichothecium roseum* into

* In contrast to rosenonolactone, desoxorosenonolactone prepared by incubation of *T. roseum* in the presence of [1-^{14}C]acetate could not readily be purified to radiochemical purity.

[10] A. J. BIRCH, R. W. RICKARDS, H. SMITH, A. HARRIS and W. B. WHALLEY, *Proc. Chem. Soc.* 223 (1958); *Tetrahedron* 7, 241 (1959).
[11] J. J. BRITT and D. ARIGONI, *Proc. Chem. Soc.* 224 (1958).
[12] B. ACHILLADELIS and J. R. HANSON, *Tetrahedron Letters* 4397 (1968).
[13] B. ACHILLADELIS and J. R. HANSON, *Tetrahedron Letters* 1295 (1967); *Phytochem.* 7, 589 (1968).
[14] W. CHARNEY and H. L. HERZOG, *Microbial Transformations of Steroids*, p 670, Academic Press, New York (1967).

rosenonolactone (II) and rosololactone (I) to the extent of 1·4 and 1·9 per cent respectively. Ozonolysis of the vinyl groups of both the starting desoxorosenonolactone and the derived rosololactone gave formaldehyde samples containing the same fraction (one-eighth, cf. Ref.[10]) of the respective diterpene radioactivity. Thus specific conversion of the intact diterpene has occurred, rather than incorporation of label only after extensive oxidative degradation.

The relative yields of these diterpenes at different stages of growth of the organism (Fig. 1) are in agreement with this conversion. Desoxorosenono-, rosenono- and rosolo-lactones are produced mainly during the log-phase of the growth of *T. roseum* in shaken culture. The yield of desoxorosenonolactone, however, decreases towards the end of the log-phase while that of rosenono- and rosolo-lactones continues to increase. Assuming that the rosenono- and rosolo-lactones are entirely derived from the desoxorosenonolactone, this suggests that the rate of synthesis of desoxorosenonolactone is faster than its oxidation rate during the early stages of the log-phase, but that the relative rates are reversed towards the end of the log-phase.

EXPERIMENTAL

Radioactivity was assayed as described by Birch *et al.*[15] on infinitely thick samples to an error of ± 3 per cent by a thin-window Geiger system. Relative molar activities (r.m.a.) are as previously defined,[15] and absolute activities where required were obtained by calibration against a ^{14}C-perspex reference source.

Isolation of Metabolites

Trichothecium roseum Link (I.C.I. Nobel Division strain F227) was cultured at 25° in the dark in 750 ml conical flasks (containing 225 ml of medium prepared as described[2]) on a rotary shaker (175 rev/min). A thick mycelial suspension formed within 4 days after inoculation with a standard spore suspension. For production studies, three flasks were harvested at intervals. The washed and dried mycelia were powdered and extracted in a Soxhlet with ether for 6 hr. The culture fluid was extracted with ether, and the combined either extracts were evaporated and chromatographed on alumina (50 g, Peter Spence grade "H"). After development with petroleum ether, elution with benzene–petroleum ether (1:1) gave desoxorosenonolactone (III). Further elution with $CHCl_3$ yielded rosenonolactone (II), and with $CHCl_3$–methanol (20:1) gave rosololactone (I). Metabolites were identified, after crystallization, by m.p., mixed m.p. and i.r. spectra in comparison with authentic samples. Variations in the yield of desoxorosenonolactone and the combined yield* of rosenono- and rosolo-lactones with incubation time are shown in Fig. 1.

^{14}C-*Rosenonolactone*

Addition of sodium [1-^{14}C]acetate (300 μc) to three flasks of *T. roseum* after 5 days' growth gave, after a further 3 days, ^{14}C-rosenonolactone (120 mg; r.m.a. $15{\cdot}8 \times 10^6$, 4·62 μc, 1·5 per cent incorporation).

^{14}C-*Desoxorosenonolactone*

^{14}C-Rosenonolactone (100 mg) and hydrazine hydrate (0·6 ml, 99 per cent) in refluxing ethanol (2 ml) for 2 hr gave ^{14}C-*rosenonolactone hydrazone* (89 mg), m.p. 161–162° from aqueous methanol (Found: C, 72·6; H, 9·3; N, 8·3. $C_{20}H_{30}N_2O_2$ required: C, 72·7; H, 9·2; N, 8·5 per cent).

The hydrazone (240 mg) and KOH (180 mg) in diethylene glycol (4 ml) containing hydrazine hydrate (0·2 ml, 99%) were heated on the steam-bath for 7 min, distilled until the temperature reached 175°, and then refluxed at this temperature for 15 min. Chromatography of the neutral product on alumina and elution with benzene–petroleum ether (1:1) afforded ^{14}C-desoxorosenonolactone (127 mg), m.p. 118–119°, $[\alpha]_D + 60°$ (*c* 0·2 per cent in EtOH), (lit.[4] m.p. 115–116°, $[\alpha]_D + 57°$ in $CHCl_3$), (r.m.a. $15{\cdot}7 \times 10^6$) (Found: C, 79·3; H, 10·2. Calc. for $C_{20}H_{30}O_2$: C, 79·4; H, 10·0 per cent).

This ^{14}C-desoxorosenonolactone on ozonolysis in acetic acid (distilled from CrO_3) followed by reduction of the ozonide with zinc dust gave formaldehyde, isolated as the 2,4-dinitrophenylhydrazone, m.p. 163–165° (r.m.a. $1{\cdot}98 \times 10^6$, 12·6 per cent of the desoxorosenonolactone radioactivity).

* Although the individual yields of rosenono- and rosolo-lactone varied considerably in different experiments, the combined yield was reproducible.

[15] A. J. Birch, R. A. Massey-Westropp, R. W. Rickards and H. Smith, *J. Chem. Soc.* 360 (1958).

Microbiological Oxidation of ^{14}C-Desoxorosenonolactone

^{14}C-Desoxorosenonolactone (30 mg, 1·2 μc) in acetone (0·6 ml) was distributed equally between three flasks of *T. roseum* after 4 days' growth as above. At harvest after a further 3 days were obtained (i) ^{14}C-desoxorosenonolactone (65 mg), m.p. 114–116° (r.m.a. $4{\cdot}20\times10^6$, 0·70 μc, 58 per cent recovery of added ^{14}C-desoxorosenonolactone), (ii) ^{14}C-rosenonolactone (133 mg), m.p. 213–214° (lit.[6] m.p. 214°) (r.m.a. $51{\cdot}8\times10^3$, 0·017 μc, 1·4 per cent incorporation), and (iii) ^{14}C-rosololactone (136 mg), m.p. 186° (lit.[3] m.p. 186° (r.m.a. $69{\cdot}3\times10^3$, 0·023 μc, 1·9 per cent incorporation).

Ozonolysis of this ^{14}C-rosololactone gave formaldehyde isolated as the 2,4-dinitrophenylhydrazone (r.m.a. $8{\cdot}51\times10^3$, 12·3 per cent of the rosololactone radioactivity).