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THE METHYL MIGRATION IN ROSENONOLACTONE BIOSYNTHESIS*

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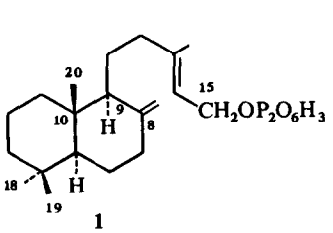
Key Word Index—*Trichothecium roseum*; rosenonolactone; diterpenoid; biosynthesis.

Abstract—The C-10 to C-9 methyl group migration in rosane biosynthesis proceeded without loss of mevalonoid hydrogen from C-20 thus excluding a cyclopropane intermediate from the biosynthesis sequence. 10β,19-Dihydroxy-rosa-15-ene was comparatively efficiently incorporated into rosenonolactone but it could not be detected in the fermentation.

INTRODUCTION

The migration of C-20 from C-10 to C-9 is a common feature of the biosynthesis of diterpenoids. In the bicyclic series it is exemplified by the clerodanes and in the tricyclic series by the rosanes and compounds such as rimuene and dolabradiene. In the bicyclic series the rearrangement of C-20 to C-9 leads to the generation of a C-10 carbonium ion. This is followed by a hydride shift from C-5 to C-10 and the migration of C-19 from C-4 to C-5 with the eventual discharge of the carbonium ion by olefin formation or by the attack of an oxygen nucleophile on ring A. In many cases there is a *trans:anti:-trans* relationship between the migrating groups suggestive of a concerted process. However the sequence of events is less clear with the rosane lactones. The 19 → 10 lactone ring of rosenonolactone (3) occupies the same (β) face of the molecule as the methyl group which has undergone rearrangement. This suggests that lactonization and methyl group migration are not concerted processes. The *pro*-4*R*-mevalonoid hydrogen atom, originally at C-9 in the labdadienol pyrophosphate (1) precursor and *trans* to C-20, has migrated to C-8 [1]. However rosenonolactone retains mevalonoid hydrogen

* Part 22 in the series 'Studies in Terpenoid Biosynthesis'. For part 21 see Dockerill, B. and Hanson, J. R. (1978) *Phytochemistry* 17, 701.



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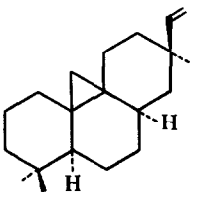

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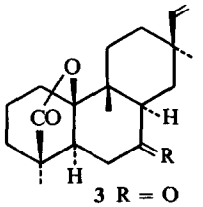

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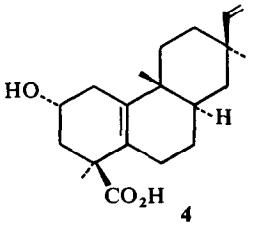
7 R = H_2



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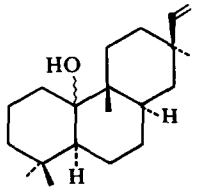

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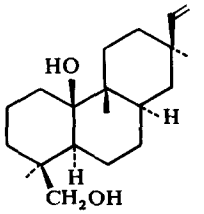

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at C-1,C-5 and C-6 thus excluding the discharge of the C-10 carbonium ion through olefinic intermediates involving these centres. Bicyclic labdanes in which C-19 has already been oxidized, and a hydrocarbon, pimara-8(9),15-diene which occurs in *T. roseum* [2], are not incorporated into the rosanes. Bearing in mind the intervention of the triterpene, cycloartenol, in algal and plant sterol biosynthesis [3], the C-9, C-10, C-20-cyclopropane (**2**) seemed a plausible intermediate. Once the intermediate was formed, oxidation could then proceed at C-19. Acid-catalysed cleavage of the cyclopropane ring would then generate an electrophilic centre at C-10 and hence lactonization would proceed. In this paper we report some experiments concerned with this stage in the biosynthesis.

RESULTS AND DISCUSSION

The methyl group, C-20, is derived from the C-3′ methyl group of mevalonate [1,4–6]. A cyclopropane ring involving this centre would entail the loss of mevalonoid hydrogen. MVA-[methyl-^{3}H, ^{14}C] (^{3}H: ^{14}C, 11.85:1) [7, 8] was fed to *Trichothecium roseum* and the rosenonolactone (**3**, ^{3}H: ^{14}C, 10.95:1 0.1% incorporation) was isolated. The ^{3}H:^{14}C ratio corresponds to the retention of 11 out of the possible 12 tritium atoms. Since one tritium atom is lost in the formation of the 8(17)-double bond of the labdadienol pyrophosphate (**1**), no further tritium can be lost in the formation of rosenonolactone thus excluding a cyclopropane intermediate such as **2**. Hence the rearrangement step is probably a simple 1:2 methyl migration.

In an effort to detect free diterpenoid acidic metabolites, labdadienol-[15-^{3}H] pyrophosphate (**1**) [1] was fed to *T. roseum*. After three days growth, the acidic fraction contained only 0.0006% of the initial radioactivity. We have been unable to detect isorosenolic acid (**4**) [9] in our strain of the fungus. However, in view of the retention of mevalonoid hydrogen at C-5 [1] in rosenonolactone, compounds of this type are unlikely to be intermediates in the biosynthesis.

A plausible intermediate in the biosynthesis could be 10-hydroxy rosa-15-ene(**5**) which then undergoes sequential oxidation at C-19. A case can be made for either stereochemistry, *α-trans* to C-10, *β-cis* to C-19. 10*β*, 19-Dihydroxyrosa-15-ene-[1,7,12,18-^{14}C] (**6**) [10] was prepared by LAH reduction of desoxyrosenonolactone-[1,7,12,18-^{14}C] (**7**), itself prepared biosynthetically from MVA-[2-^{14}C]. The labelled diol was incorporated into rosenonolactone to the extent of 0.63% (cf. labdadienol-[15-^{3}H] pyrophosphate, 0.16% [11]; desoxyrosenonolactone 1.4% [12] and 3.5% [1]). However, although this incorporation was of an appropriate level, we were unable to detect the diol in the broth by either GLC or dilution analysis. Hence this incorporation can only at present have the status of a microbiological transformation. Nevertheless the comparative efficiency of the incorporation does suggest that the biosynthesis might involve a 10-alcohol or enzyme-bound intermediate which is sequentially oxidized at C-19 in a bound form. The lactonization step would then represent the final displacement of the metabolite from the enzyme system.

EXPERIMENTAL

General experimental details have been described previously [2]. *Trichothecium roseum*, strain CMI 50 600, was used in this work.

Incubation with MVA-[3′-^{14}C, 3′-^{3}H]. The mevalonate (2 μC- of ^{14}C, ^{3}H: ^{14}C, 11.85:1) in H_2O (0.3 ml) was fed to a 3-day-old culture (100 ml) of *T. roseum*. After a further 8 days, the metabolites were isolated and purified by PLC on Si gel to afford rosenonolactone (10.3 mg) which was recrystallized to constant radioactivity. It has ^{3}H· ^{14}C, 10.95:1; 0.1% incorporation.

*Incubations with labda-*8(17),13-*dien*-15-*ol*-[15-^{3}H] *pyrophosphate.* (a) *Isolation of the acidic metabolites.* The pyrophosphate (37.2×10^6 dpm) was distributed between 3 one-day-old cultures (100 ml) of *T. roseum*. The cultures were extracted with EtOAc after a further 3 days growth. The mycelium was stirred with 10% NaOH (150 ml) for 2 hr. After filtration this soln was acidified (conc HCl), and extracted with EtOAc The extracts were combined, concd to 40 ml and extracted with satd. aq. $NaHCO_3$ (3 × 15 ml). These extracts were washed with EtOAc, acidified (2N HCl) and the acidic metabolites recovered in EtOAc. The extract was dried and the solvent evapd. The residue was taken up in EtOAc (1.0 ml) and an aliquot (0.1 ml) counted. It showed 23.2 dpm corresponding to 0.0006% of the initial radioactivity.

(b) *Attempted identification of* 10*β*,19-*dihydroxyrosa*-15-*ene.* The pyrophosphate (37.2×10^6 dpm) was distributed between 3 one-day-old cultures of *T. roseum*. The cultures were extracted after a further 3 days growth. The diol (20 mg) was added to the crude extract which was then purified by PLC on Si gel in EtOAc–petrol (2:3). The diol was recovered and recrystallized from petrol; after 2 recrystallizations it showed only background radioactivity. (c) The extract from 10 six-day-old cultures was examined by GLC on a 1% OV-17 column at 230° (N_2 carrier gas 50 ml/min). The R_f of the diol under these conditions was 2.6 min. No peaks of a similar R_f were observed in the *T. roseum* extract.

Preparation of desoxyrosenonolactone [1,7,12,18-^{14}C]. MVA-[2-^{14}C] (25 μCi) in H_2O (0.2 ml) was fed to a culture of *T. roseum* immediately after inoculation. The metabolites were recovered after 4 days growth and purified by PLC on Si gel in EtOAc–petrol (2:3) to afford desoxyrosenonolactone-[1,7,12,18-^{14}C] (3.2 mg), mp 117–118°, (2.14×10^5 dpm, 0.4% incorporation).

Preparation of 10*β*,19-*dihydroxyrosa*-15-*ene.* The lactone from the above expt, was treated with LAH (5 mg) in Et_2O (10 ml) for 2 hr. EtOAc (5 ml) followed by 2N NaOH (10 ml) was added. The product was recovered in EtOAc and purified by PLC on Si gel in EtOAc–petrol (2:3) to afford the diol (1.0×10^5 dpm). The diol had mp 144–145° (lit., 146° [10]). NMR: δ 0.82, 0.92 and 1.02 (each 3H, *s*), 3.18 and 3.60 (each 1H, AB *q*, J = 12 Hz) 4.85 (2H, *m*), 5.80 (1H, *m*), (determined on an inactive sample).

Incorporation of 10*β*,19-*dihydroxyrosa*-15-*ene into rosenonolactone.* The diol (1.0×10^5 dpm) in EtOH (0.3 ml) and 10% Tween 80 (0.3 ml) was added to a freshly inoculated culture of *T. roseum* (100 ml). The culture was extracted after 10 days growth to afford rosenonlactone (12 mg) which was crystallized from EtOAc to constant radioactivity, 53.4 dpm/mg, 0.63% incorporation.

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