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THE BIOSYNTHESIS OF OVALICIN: ISOLATION OF β-trans-BERGAMOTENE[1]

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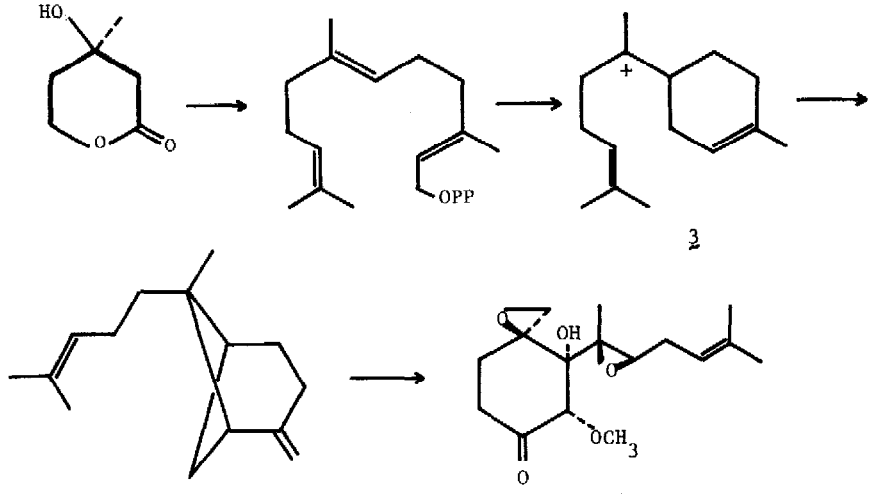
Recent work in this laboratory[2] and elsewhere[3] has implicated the sesquiterpene hydrocarbon β-trans-bergamotene (1) in the biosynthesis of the immunosuppressive antibiotic ovalicin[4] (2). Incorporation experiments using both [3,4-$^{13}C_2$] mevalonate and [1,2-$^{13}C_2$]-acetate have supported a rearrangement pathway involving 1,3-migration of the eight-carbon side chain of the bisabolyl cation 3 formed by cyclization of farnesyl pyrophosphate. β-Bergamotene was postulated to be a likely intermediate in this rearrangement pathway[5]. We now wish to report the isolation and identification of β-trans bergamotene from mycelial extracts of Pseudorotium ovalis.



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Cultures of P. ovalis were grown under the usual conditions[2,4] until mycelial pigmentation was first noticeable (6-7 days). The mycelium was collected by filtration, dried, and extracted with n-pentane in a Soxhlet ap-

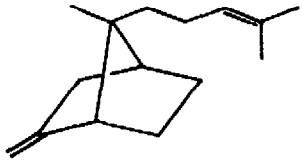
paratus for two days. The concentrated extract was eluted with pentane through silica gel to remove polar constituents and then further purified by preparative layer chromatography (silica gel/pentane). The major hydrocarbon component, A, (R_f = 0.6) was shown to be >95% pure by glc analysis (r.t. 5.5 min, 6 ft 5% Carbowax, 120°). Typically, 2-4 mg of A could be isolated from a 6 ℓ. culture. Reduction of aeration by the use of slower shaking speeds (180 rpm) and larger culture volumes per flask (e.g. 1100 ml/2800-ml Fernbach) appeared to favor higher titers of A.

The spectroscopic data for A are completely consistent with structure 1 for β-trans-bergamotene: nmr($CDCl_3$) δ 0.70 (s, $C\underline{H}_3$, 3H), 1.60 (s, $C=CC\underline{H}_3$, 3H) and 1.69 (s, $C=CC\underline{H}_3$, 3H) superimposed on 1.25 - 2.7 (m, 12H), 4.57 (bs, $=C\underline{H}_2$, 2H)[6], and 5.16 (t,J=8 Hz, $=C\underline{H}$, 1H); i.r. $\lambda_{max}^{CCl_4}$ 3080, 1645, 1360, 875 cm^{-1}; m/e (70 ev) (rel. int.) 204(7), 189 (1.5), 161 (13), 135 (4), 133 (17), 94 (13), 93 (62), 92 (20), 91 (25), 79 (32), 77 (22), 69 (100). Direct comparison of the nmr and ir spectra of A with those of synthetic 1[7] showed the two compounds to be identical in all respects.

The structure 1 had previously been assigned to a bicyclic sesquiterpene isolated from the root oil of Valeriana wallichii[8]. When total synthesis of both β-cis[9] and β-trans-bergamotene[7] demonstrated that this assignment was wrong, the structure of the Valerian sesquiterpene was revised to sesquifenchene (4). This latter assignment was later confirmed by total synthesis[10]. The isolation of authentic β-trans-bergamotene from P. ovalis is therefore the first verified occurence of this sesquiterpene in a natural source and supports the suggested intermediacy of this substance in the biosynthesis of ovalicin.[11,12]

In our earlier papers[2] we suggested that bergamotene might be oxidatively cleaved to the tetraene 5, which in turn would undergo oxidation to form ovalicin. An authentic sample of 5 was therefore synthesized by a route based on Corey's synthesis of fumagillin[13,14] and the mycelial extracts of P. ovalis analyzed by glc for the presence of natural tetraene (r.t. 6.9 min). Within the limits of

detection (ca. 10μg/1ℓ. culture) no tetraene was evident. This substance is therefore either not produced at all by P. ovalis or is present at extremely low levels.



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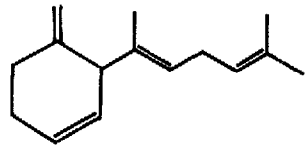

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Incorporation experiments designed to test the intermediacy of bergamotene in the biosynthesis of ovalicin are in progress[15].

References and Notes

1. Presented at the U.S.-Japan Seminar on the Biosynthesis of Natural Products, sponsored by the National Science Foundation and the Japan Society for the Promotion of Science, Honolulu, Hawaii, June 14-18, 1976.
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11. Nozoe has also isolated bergamotene from Aspergillus fumigatus, a fungus which produces fumagillin[13], a substance closely related to ovalicin. Reported at the U.S.-Japan Seminar on the Biosynthesis of Natural Products. *See the accompanying communication.

12. β-Bergamotene may also be present as a minor constituent of opopanax oil. Cf. J. A. Wenninger and R. L. Yates, J. Ass. Offic. Anal. Chem., 52, 1155 (1969).

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15. Details of the synthesis of 5 will be published at a later date along with the results of incorporation experiments involving labeled 5.

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