Structures of the Mating-type-specific Prohormones of Mucorales

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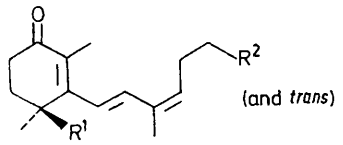
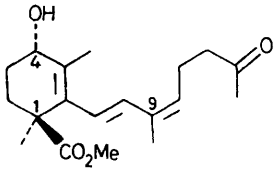
Summary Specific prohormones from separate mating types of *Blakeslea trispora* are shown to have structures (**3**) (from *plus*) and (**6**)—(**9**) (from *minus*); each is specifically produced by one mating-type and only converted into the active hormones (**1**) and (**2**) by the other.

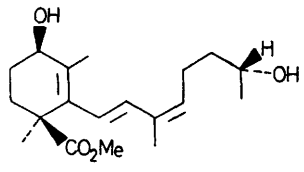
IN heterothallic mucoraceous fungi,[1,2] isolated mycelia of *plus* or *minus* mating-type produce small amounts of specific 'prohormones' conveniently termed P^+ and P^- respectively,[3,4] such that only mycelium of the opposite mating-type will convert them into trisporic acids (**1**,**2**) which are the true hormones eliciting sexual differentiation in both mating-types.[1,5] We report chemical characterizations of the prohormones. Culture media[6] from *plus Blakeslea trispora* afforded 2—4 mg/l of a complex mixture of neutral Et_2O soluble metabolites. Direct assays against *minus Mucor mucedo*[5] of silica gel scraped from t.l.c. of the mixture localized active prohormone in one zone, with R_f and u.v. absorption agreeing with data [3,4] for P^+. From 15 l of culture medium, 1·6 mg of P^+ was purified and characterized as (**3**): $C_{19}H_{28}O_4$ by high-resolution m.s.; perturbed triene u.v. absorption [λ_m(Et_2O) 276(sh)283 (log ϵ 4·18), 296 (sh) nm, unchanged by $NaBH_4$]; i.r. ν_{max} ($CHCl_3$) 1725 (s, br: ester + saturated ketone >C = O), 3460 cm^{-1} (br: HO); M^+ m/e = 320, changed to 322 by $NaBH_4$, to 362 by Ac_2O–pyridine, and to 334 by transesterification with NaOEt. The n.m.r. spectrum of P^+ was fully interpretable in terms of the predominantly 9-*cis*-structure (**3**) by reference to measurements for the known methyl 9-*cis*- and 9-*trans*-trisporate B (**4**) and for the $NaBH_4$ reduction product, mainly (**5**) (see below) from methyl trisporate C. The stereochemistry of (**3**) at C-1 follows from that of (**2**)[7] and at C-4 is tentatively assigned from m.s. data: given the preferred conformation of methyl trisporate C,[7] the preferred product of its $NaBH_4$ reduction is predictably[8] (**5**) in which the possibility of H-bonding between the *cis*-1,4 ester and OH groups brings the latter into an axial arrangement favouring dehydration; in the mass spectrum of (**5**) (and of related compounds), loss of the 4-OH as water is a very favoured process, whereas in the m.s. of natural



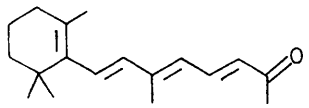
(1) $R^1 = CO_2H$ $R^2 = Ac$
(2) $R^1 = CO_2H$ $R^2 = CHOHMe$
(4) $R^1 = CO_2Me$ $R^2 = Ac$
(6) $R^1 = CH_2OH$ $R^2 = Ac$
(7) $R^1 = CH_2OH$ $R^2 = CHOHMe$
(8) $R^1 = Me$ $R^2 = Ac$
(9) $R^1 = Me$ $R^2 = CHOHMe$



(3)



(5)



(10)

P+ this process is relatively much less prominent. The epimeric configuration shown in (**3**) is therefore suggested.

Similar work on the complex of neutral metabolites from *minus B. trispora* confirmed that materials with P− prohormone activity occur in two well-separated chromatographic regions,[3] both heterogenous. From the more polar zone we obtained the trisporols (**6**, **7**) and from the less polar zone the corresponding *gem*-dimethyl compounds (**8**, **9**). These are all known compounds[5] and were characterized by u.v., m.s., and chromatographic data. In bioassays (with *plus M. mucedo*) the relative activities of (**6**), (**7**), and (**8** and **9**) are approximately as 50:10:1.

Labelling experiments confirm that the prohormones, like (**1**) and (**2**) in mixed *plus-minus* cultures,[9] are formed *via* the C-18 ketone (**10**). In the single strains, the overall synthesis of (**1**, **2**) is repressed, but some steps still occur though at a minimal (un-induced, or 'gratuitous') level. We have shown[2] that distinctive features in the resulting complex mixtures of minor metabolites arise because the pattern of gratuitous reactions is different in the two mating-types. The structures now assigned to the active components of these mixtures, (**3**) in *plus* and (**6**)—(**9**) in *minus*, accord with that analysis since both their specific production from (**10**) by one mating-type and their specific transformation into (**1**) and (**2**) by the other are consistent with what is known [2,5] about the patterns of the mating-type-linked gratuitous reactions.

Recent observations[10] on some Mucor species in which the copulatory outgrowths are aerial suggested that the volatile 'zygotropic hormones' of these species are in effect the P+ and P− prohormones. This too accords with the structures now assigned, since all are much less polar than the hormones proper, (**1**) and (**2**), and their volatility at room temperature, though slight, is experimentally demonstrable.

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[1] G. W. Gooday, *Soc. Gen. Microbiol. Symp.*, 1973, **23**, 259.
[2] J. D. Bu'Lock, B. E. Jones, S. A. Quarrie, and N. Winskill, *Naturwiss.*, 1973, **60**, 550.
[3] T. A. Werkman and H. van den Ende, *Arch. Microbiol.*, 1973, **90**, 365.
[4] R. P. Sutter, D. A. Capage, T. L. Harrison, and W. A. Keen, *J. Bact.*, 1973, **114**, 1074; the P,π and M,μ of these authors are equivalent to the P+ and P− of ref. 3.
[5] J. D. Bu'Lock, D. Drake, and D. J. Winstanley, *Phytochem.*, 1972, **11**, 2011.
[6] J. D. Bu'Lock and D. J. Winstanley, *J. Gen. Microbiol.*, 1971, **69**, 391.
[7] J. D. Bu'Lock, D. J. Austin, G. Snatzke, and L. Hruban, *Chem. Comm.*, 1970, 255.
[8] G. Toromanoff, *Topics in Stereochemistry*, 1967, **2**, 157.
[9] J. D. Bu'Lock, B. E. Jones, S. A. Quarrie, D. Taylor, and N. Winskill, *J. Gen. Microbiol.*, 1974, **80**, 301.
[10] D. A. Mesland, J. G. Huisman, and H. van den Ende, *J. Gen. Microbiol.*, 1974, **80**, 111.