Mechanism and Stereochemistry of the Enzymic Conversion of Prenyl to Chromen Structures, as effected by Deguelin Cyclase

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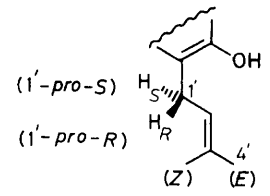
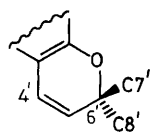
Isotopic labelling shows that enzymic removal of the 1′-*pro-S*-hydrogen from an *o*-prenylated phenol correlates with the *E*-methyl of the latter becoming the *pro-R*-methyl at 6′ of the chromen produced; overall, the reaction is of limited stereoselectivity as deguelin cyclase does not specifically recognise the 1′-*pro-S*- or *pro-R*-hydrogens for removal.

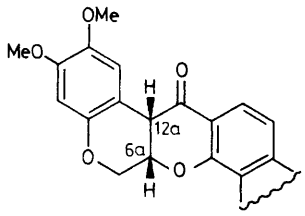
The 2,2-dimethylchromen ring system is found in many types of natural product and our earlier work using a cyclase enzyme isolated from *Tephrosia vogellii*[1] has shown that 6a*S*,12a*S*-rotenonic acid (**1a**) is converted into 6a*S*,12a*S*-deguelin (**2a**) with no evidence of a readily isolatable intermediate. Cyclisation *via* a dienone could be consistent with these findings. During the cyclisation process three stereochemical changes occur: the 1′-prochiral centre of rotenonic acid (**1a**) is extinguished, the (*Z*)-(*E*)-relationship of its 4′,5′-methyls is dissolved, and a new prochiral centre at C-6′ of the chromen is created. We have shown[2] that the (*E*)-methyl (C-4′) of rotenonic acid becomes dominantly the 8′-(*pro-R*) or α-methyl of deguelin, but only to the extent of 73% in an unstereospecific process. Using the synthetic work of the



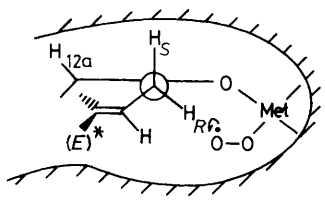
(1)



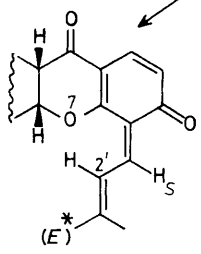
(2)



(α)



(11)



(12)

$$\mathrm{Lig_NMet^N + ArOH \rightleftharpoons Lig_{N-1}Met^NOAr + Lig^- + H^+}$$

$$\mathrm{Lig_{N-1}Met^NOAr + O_2 \rightleftharpoons Lig_{N-1}Met^{N+1}(\dot{O}_2)OAr} \quad (3)$$

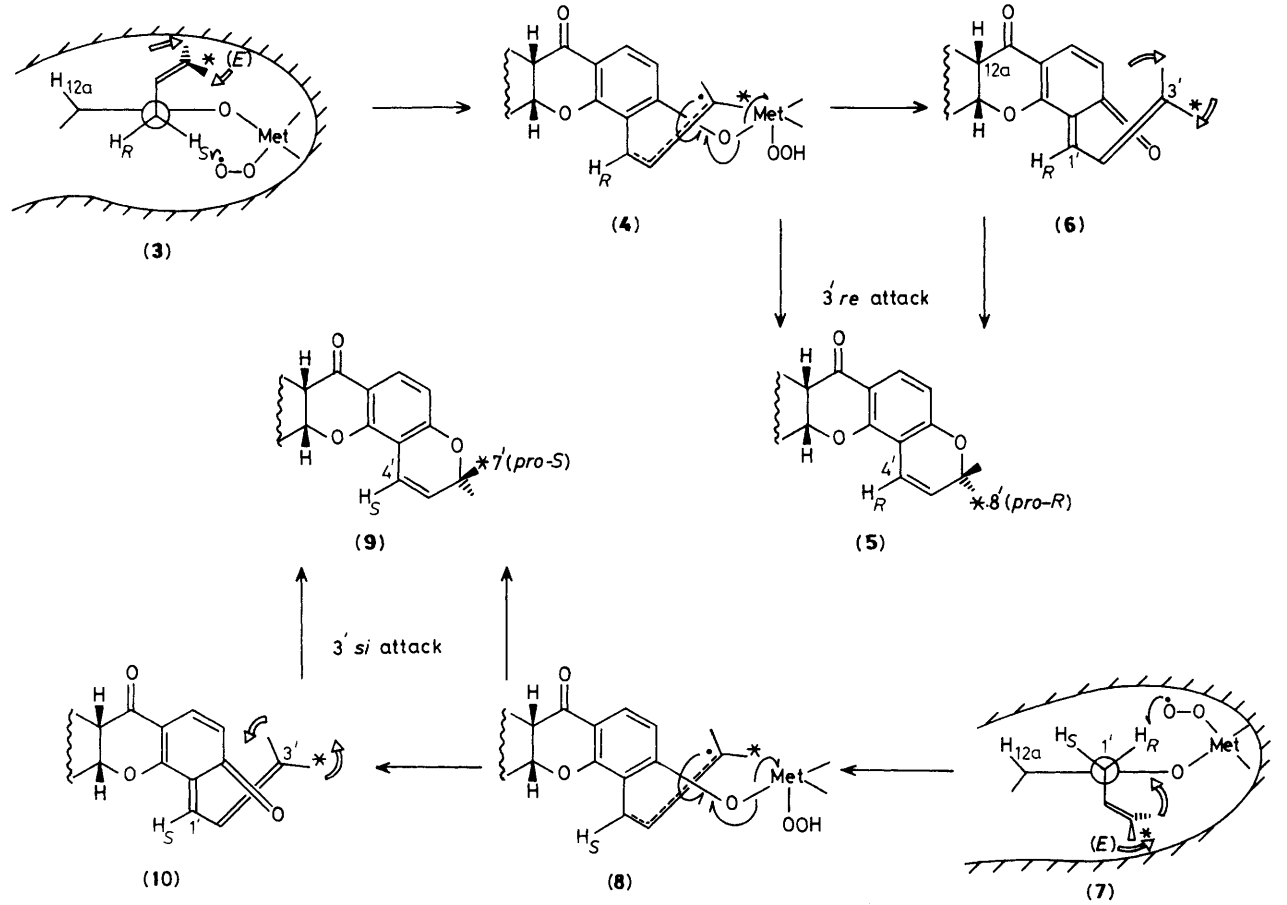
$$\mathrm{(3) \rightarrow (4) \rightarrow (5) + HOOMet^NLig_{N-1}}$$

$$\mathrm{HOOMet^NLig_{N-1} + Lig^- + H^+ \rightarrow H_2O_2 + Lig_NMet^N}$$

Scheme 1

preceding Communication[3] we now report on the fate of the prochiral hydrogens at C-1′ in rotenonic acid during the chromen cyclisation.

Each 1′-tritiated dimethylallyl phenol (**1a**), *R* or *S*, was mixed with [4′-^{14}C]-rot-2′-enonic acid, to give a predetermined ^{3}H/^{14}C ratio, and incubated with a *T. vogellii* cyclase enzyme preparation. The deguelin produced was isolated by h.p.l.c. and its ^{3}H/^{14}C ratio determined. For the 1′*R*-[1′-^{3}H]-rotenonic acid supplied at ^{3}H/^{14}C = 3.56, the deguelin isolated in two experiments had ^{3}H/^{14}C ratios of 3.25 and 3.17 (mean 3.21), the completeness of the reaction (judged from the [^{14}C] count of unused rotenonic acid) being 96.0 and 97.3%. This indicates a relative ratio of *S*-^{1}H/*R*-^{3}H bond breaking of 90.2/9.8, but the selectivity is of course exaggerated because of isotope effects on the *R*-^{3}H cleavage. Thus when 1′-*S*-[1′-^{3}H]-rotenonic acid was supplied to the enzyme at ^{3}H/^{14}C = 4.44, the deguelin isolated in two experiments had ^{3}H/^{14}C ratios of 2.52 and 2.35 (mean 2.44), the completeness of the reactions being 99.1 and 99.4%. This indicates relative rates of *S*-^{3}H/*R*-^{1}H bond breaking to be 45/55; in this case the rate of *S*-^{3}H bond breaking is reduced by the ^{3}H isotope effect such that it is just slower than *R*-^{1}H removal. Using these data, and



Scheme 2. Models for the enzyme formation of chromens.

assuming that the kinetic isotope effect is the same for both the 1′-hydrogen positions, it can be calculated that the relative rates of *pro-S*-^{1}H removal/*pro-R*-^{1}H removal (*i.e.*, the stereochemical preference) is 73.1/26.9 and that a ^{3}H-isotope effect of 3.3 operates.

Our deguelin cyclase enzyme has been partially purified by successive ammonium sulphate precipitation, Sephadex G25 chromatography, h.p.l.c. size exclusion (Protein PAK 300W), and h.p.l.c. ion exchange (DEAE-50W), to four bands on gel electrophoresis. The major features of mechanistic interest are that the enzyme requires oxygen but no added cofactors even after purification. The presence in the purified protein of both iron and copper was indicated by atomic absorption spectroscopy and the need for metal ions was shown by inhibition by *o*-phenanthroline and other chelators counteracted by added iron(II) or copper(II) ions. A tentative mechanistic proposal is as in Scheme 1. It is possible that the radical (**4**) could decompose to a dienone (**6**) before cyclisation. The stereochemical situation thus becomes as shown in Scheme 2. Removal of H_S in (**3**) having (*E*)-labelling leads to radical (**4**), or in the limit dienone (**6**). 3′-*re*-Attack with clockwise rotation of the methyls then leads to chromen (**5**) carrying H_R and with labelling in the 8′(*R*)-methyl; the 73% loss of H_S correlates with the 73% attainment of 8′(*R*)-methyl labelling. Similarly, removal of H_R in (7) having (*E*)-labelling leads to (**8**) or (**10**), 3′-*si*-attack with anticlockwise rotation of the methyls giving chromen (**9**) carrying H_S and labelling in the 7′(*S*)-methyl. Removal of H_S in conformation (**11**) leads to a radical or dienone (*cf.* **12**) unsuitable for chromen cyclisation without further bond rotations. In addition steric compression between O-7 and the 2′-hydrogen inhibits the attainment of fully stabilised planar forms.

Deguelin cyclase is thus a mechanistically 'untidy' enzyme and is not evolutionarily perfected in the sense of giving specific recognition to H_R or H_S for removal, and hence not leading to an (*E*)- or a (*Z*)-methyl becoming entirely oriented *pro-R*- or *pro-S* in the product. In one sense, of course, such stereochemical issues have no significance to the plant in terms of the nature of the product and its precursor. The exact stereochemistry of action of the cyclase enzyme appears to depend on local factors within its own structure, and evolutionary pressure seems not to have necessitated modification leading to a more precise sharpening of stereochemical attack.

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