Biosynthesis of Mycophenolic Acid. Oxidation of 6-Farnesyl-5,7-dihydroxy-4-methylphthalide in a Cell-free Preparation from *Penicillium brevicompactum*

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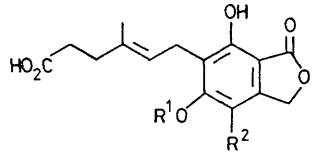
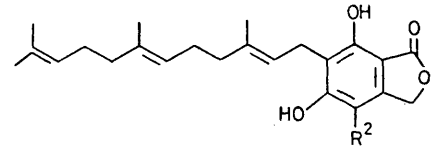
Summary The conversion of 6-farnesyl-5,7-dihydroxy-4-methylphthalide (**5**) into mycophenolic acid (**1**) proceeds through the hydroxy-ketone (**11**) by oxidation of the central double bond.

IN the biosynthetic pathway to mycophenolic acid (**1**) 5,7-dihydroxy-4-methylphthalide (**7**), 6-farnesyl-5,7-dihydroxy-4-methylphthalide (**5**), and normethylmycophenolic acid (**2**) take part as sequential intermediates.[1] With respect to the mechanism that leads from (**5**) to (**2**) and to (**1**), it has been reported[2] that mycophenolic and normethylmycophenolic aldehydes are not intermediates. In order to investigate this mechanism we have prepared a total enzymic extract from *P. brevicompactum* that can lead not only to prenylation of the aromatic nucleus of (**7**), using farnesyl pyrophosphate (**10**)[3] as recently reported[4], but can also lead to oxidation of the central double bond of the farnesyl chain of (**5**). *P. brevicompactum* was grown on a Czapek–Dox medium in a shaken culture for 72 h at 25 °C, and the mycelium was homogenized at 4 °C in a Potter Elvehjem homogenizer in 0·1 M phosphate buffer (pH 7), containing $MgCl_2$ (0·1 M). The filtered homogenate was centrifuged and the soluble fraction was used for the transformation of (**8**) in the presence of (**10**) and ATP (0·02 mg ml^{-1}) to produce (**6**) and (**3**). Conversions of (**8**) into (**6**) and (**3**) of 10 and 2%, respectively, were observed, following concentration of the substrate to 6 mg for each 100 ml of homogenate. In contrast, the action of the enzymatic preparation in 0·1 M citrate–phosphate buffer (pH 5) on 6-farnesyl-5,7-dihydroxy-4-methylphthalide (**5**) caused oxidation into the hydroxy-ketone (**11**) [*m/e* 336 (10·6%), 245 (12·8), 237 (100), 219 (34), 207 (64), 193 (27·7), 111 (53), and 99 (93·5)], which up to now has not been isolated.

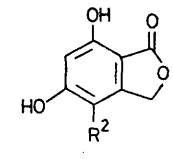
T.l.c. monitoring of this conversion shows the progressive disappearance of (**11**), together with the appearance of normethylmycophenolic acid (**2**) and mycophenolic acid (**1**). Finally, after 36 h, (**11**) and (**2**) have disappeared and only (**1**) remains. At the early stages, other compounds were detected which may be precursors of (**11**). These compounds are currently being investigated. The structure of (**11**) was confirmed by comparison with a synthetic sample.



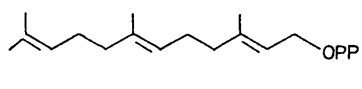
(**1**) $R^1 = CH_3$ $R^2 = CH_3$
(**2**) $R^1 = H$ $R^2 = CH_3$
(**3**) $R^1 = CH_3$ $R^2 = {}^{14}CH_3$
(**4**) $R^1 = CH_3$ $R^2 = {}^{13}CH_3$



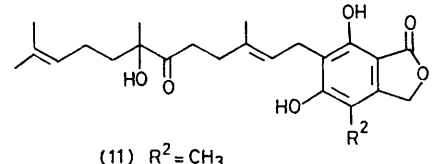
(**5**) $R^2 = CH_3$
(**6**) $R^2 = {}^{14}CH_3$



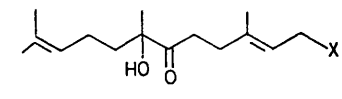
(**7**) $R^2 = CH_3$
(**8**) $R^2 = {}^{14}CH_3$
(**9**) $R^2 = {}^{13}CH_3$



(**10**)



(**11**) $R^2 = CH_3$
(**12**) $R^2 = {}^{13}CH_3$



(**13**) X = OH
(**14**) X = Br

Recently we carried out[5] the total synthesis of the acyloin derivative (**13**). Bromination of this acyloin derivative with CBr_4 and Ph_3P in MeCN[6] provided (**14**). Reaction of the bromide (**14**) with (**7**) in the presence of Ag_2O, in dioxan solution[7] provided (**11**), whereas reaction with the [^{13}C]-methyl-phthalide (**9**) gave (**12**). Administration of (**12**) to the *in vivo* culture led specifically to the formation of mycophenolic acid (**4**) with a conversion of 45%.

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