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Analogues of Mycophenolic Acid

F. ARAGOZZINI, P. TOPPINO, AND R. CRAVERI

Cattedra di Microbiologia Industriale della Facoltà di Agraria, 20133 Milano, Italia

AND

M. G. BERETTA, B. RINDONE, AND C. SCOLASTICO

Istituto di Chimica Organica della Facoltà di Scienze, 20133 Milano, Italia

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Analogues of mycophenolic acid were produced by cultures of *Penicillium brevi-compactum* fed, respectively, with 4,6-dihydroxycoumaran-3-one, 5,7-dihydroxyindan-1-one, and 2,4-dihydroxyacetophenone.

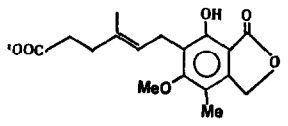
Early studies (*1*) on the ability of the enzymatic systems of *Penicillium brevi-compactum* to introduce terpenoid units into (prenylate) aromatic precursors showed that these substrates were transformed into analogues of mycophenolic acid (**1**) when an *m*-diphenol system and an aromatic carbonyl were present, in disregard of the substitution at C-4. Thus, the substrates with hydrogen, chlorine, or bromine at C-4 were prenylated, and the farnesyl chain was subsequently degraded to a C-7 unit.

The finding of mycophenolic acid together with its analogues in these experiments indicated competition between the natural biogenetic pathway and that resulting from the introduction of the modified substrate. It was still to be ascertained whether a 6/5 fused ring structure was necessary for the aromatic substrate to be incorporated and how general was the subsequent oxidative fission of the introduced prenyl side chain.

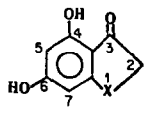
The aromatic substrates fed to *P. brevi-compactum* were 4,6-dihydroxycoumaran-3-one (**2**), 5,7-dihydroxyindan-1-one (**3**) and the monocyclic ketone 2,4-dihydroxyacetophenone (**4**).

A growing culture of *P. brevi-compactum* was fed with (**2**), and the acid metabolites were separated from the culture broth as previously described (*2*) and purified by silica-gel chromatography. Compound (**5**) was obtained from the mixture with (**1**) by tlc separation. Compound (**6**) could not be freed from mycophenolic acid. The mixture of acids was converted into the corresponding methyl ester–methyl ether mixture, which was successively analysed by glc mass spectrometry. The total yield of (**5**) and (**6**) from (**2**) was 25%. Similarly, compound (**7**) could be isolated from the transformation of (**3**), and the metabolite (**8**) was shown to be present by glc mass spectrometry of its methyl ether–methyl ester along with the analogous derivative of (**1**).

Among the transformation products from (**4**), compound (**9**) was detected, but its separation from (**1**) presented difficulties due to the presence of much mycophenolic

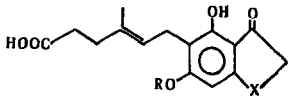


(1)



(2) X = O

(3) X = CH_2

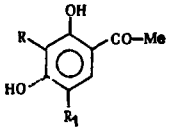


(5) X = O ; R = H

(6) X = O ; R = CH_3

(7) X = CH_2; R = H

(8) X = CH_2; R = CH_3



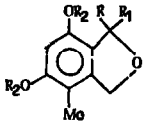
(4) R = R_1 = H

(10) R = t,t-farnesyl- ; R_1 = H

(11) R = H ; R_1 = t,t-farnesyl-

(9) R = $HO_2CCH_2CH_2C(CH_3)=CHCH_2$- ; R_1 = H

(15) R = H ; R_1 = $HO_2CCH_2CH_2C(CH_3)=CHCH_2$-



(12) R = R_1 = H ; R_2 = H

(13) R,R_1 = O ; R_2 = CH_3

(14) R = R_1 = H ; R_2 = CH_3

acid. A better yield of (**9**) and easier separation by tlc was obtained by feeding 3-*t,t*-farnesyl-2,4-dihydroxyacetophenone (**10**) to resting cells of *P. brevi-compactum*. This compound, thought to be the immediate precursor of mycophenolic acid and its analogues by oxidative loss of eight atoms of the side chain, was obtained (*3*) by reaction of 2,4-dihydroxyacetophenone (**4**) with farnesyl bromide in dioxane in the presence of Ag_2O. The isomer (**11**) was also obtained under these conditions.

A common feature of all the substrates described in this paper and in preceding communications (*1*, *2*) is the presence of an aromatic carbonyl group and two phenolic groups in the ortho and para positions. This arrangement probably is necessary for the enzymatic prenylation reaction, which invariably occurs ortho to both hydroxyls. When the carbonyl group is not present, as in 5,7-dihydroxy-4-methylphthalan (**12**), no transformation by *P. brevi-compactum* was noted. Compound (**12**) was obtained by $LiAlH_4$ reduction of 5,7-dimethoxy-4-methylphthalide (**13**) in the presence of BF_3 (*4*) to give compound (**14**), which was subsequently treated with BBr_3.

Regarding the oxidation cleavage of the prenyl side chain, it had been previously noted (*2*) that 6-geranyl-5,7-dihydroxy-4-methylphthalide was also transformed by *P. brevi-compactum* to mycophenolic acid, thus indicating good adaptability of the oxidizing enzymes and specificity for the oxidation site. The transformation of com-

pound (**11**), having the prenyl chain at C-5, to the acid (**15**) supports the belief that the tendency of the double bond to be oxidized depends upon its distance from the carbonyl group in the enzyme–substrate complex.

EXPERIMENTAL

Nuclear magnetic resonance spectra were recorded with a Varian NV-14 instrument (TMS as internal reference). Infrared spectra were measured with a Perkin-Elmer 257 spectrophotometer. Analytical glc was performed with a C. Erba Fractovap 2400 V gas chromatograph equipped with a glass column (2 m × 5 mm) packed with SE 30 on chromosorb W (oven temperature, 220°C; injector temperature, 280°C; flame ionization detector, 280°C; carrier gas N_2 at 35 ml/min); product yield was determined by using 1,1′-binaphthyl as internal standard. Mass spectra were obtained with an LKB 9000 (70 eV) glc-linked instrument. Microanalyses were obtained with a Perkin-Elmer Elemental Analyser 240.

Experiments with Growing Cells

Flasks (750 ml) containing 100 ml of medium (2% glucose, 0.2% yeast extract) were inoculated with 5% of a 60-hr submerged culture of *P. brevi-compactum* and incubated at 26°C on an alternative shaker. After 15 hr incubation, 20 mg/flask of the modified acetogenins were added (as solid if water soluble; otherwise in acetone solution). After 4 days incubation the mycelium was filtered, mixed with celite in a mortar, and extracted with ethyl acetate. The culture broth was acidified to pH 1 with concd HCl and extracted with ethyl acetate. The organic extracts were collected, washed with water until neutral, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was chromatographed on silica-gel Merck, 0.05–0.2 mm, eluting with ethyl acetate/chloroform/acetic acid 45/55/1 (ECA).

With 4,6-dihydroxycoumaran-3-one (**II**) as substrate, fractions containing (**1**) + (**5**) and (**1**) + (**6**) were collected. The mixture (**1**) + (**5**) was separated by preparative tlc (eluent: ECA; 3 runs). Compound (**5**) had mp 173–176°C (*n*-octane–ethyl acetate); λ_{max}(MeOH) 284 nm ($\varepsilon = 24\,350$); ν_{max} 1720, 1650, 1620 cm^{-1}; nmr (δ, C_5D_5N) 1.98 (3H, s, $CH_3C=$), 2.60 (4H, s, $-CH_2CH_2-$), 3.76 (2H, m, $ArCH_2C=$), 4.62 (2H, s, $-COCH_2O$), 5.86 (1H, m, $-CH=$), 6.36 (1H, s, ArH); MS (m/e) 292 (M^+ 14%), 291 (54%), 219 (100%), 179 (88%). (Found: C 61.52; H 5.51. $C_{15}H_{16}O_6$ requires: C 61.64; H 5.52.) Transformation of the mixture into methyl ester–methyl ether by excess CH_2N_2 allowed glc analysis. With 5,7-dihydroxyindan-1-one as substrate, the workup as before allowed the isolation in 8% yield of compound (**7**), mp 160–162°C (*n*-octane–ethyl acetate): λ_{max}(MeOH) 281 nm ($\varepsilon = 13\,800$); ν_{max}(nujol) 1715, 1640, 1618 cm^{-1}; nmr (δ, C_5D_5N) 1.98 (3H, s, $CH_3C=$), 2.62 (4H, s, $-CH_2CH_2-$), 2.4–2.86 (4H, m, $-COCH_2CH_2Ar$), 3.76 (2H, m, $ArCH_2C=$), 6.68 (1H, s, ArH); MS (m/e) 290 (M^+, 17%), 231 (14%), 217 (15%), 191 (33%), 177 (100%). (Found: C 66.26; H 6.31. $C_{16}H_{18}O_5$ requires: C 66.19; H 6.25.)

The fractions containing a mixture in which the analogue (**8**) was present were converted into methyl ester–methyl ether by CH_2N_2 treatment. Gas–liquid chromatographic mass spectrometry showed a 5% conversion. With 2,4-dihydroxy-

acetophenone as substrate, the analogue (**9**) was revealed (conversion 6%) by glc mass spectrometry of the methyl ester–methyl ether mixture.

Experiments with Resting Cells

The mycelium obtained by filtration from 10 flasks after 48 hr of growth was washed three times with sterilized water and divided into 10 flasks (750 ml) filled with 100 ml water.

Compound (**10**) dissolved in 1 ml of acetone was added, and the transformation required 8 days under the usual conditions. After usual workup, tlc (ECA) showed excess of (**9**) over (**1**). The residue, 180 mg, was chromatographed on 18 g of silica-gel Merck 0.05–0.2 mm (eluent ECA); the fractions containing (**9**), 40 mg, were further purified by preparative tlc (eluent benzene/methanol 9/1). Pure (**9**) (20 mg) was thus obtained: mp 125–128°C (*n*-octane–ethyl acetate); λ_{max}(MeOH) 232 nm ($\varepsilon = 23\,400$), 281 nm ($\varepsilon = 29\,500$) and 315 nm ($\varepsilon = 13\,450$); ν_{max} 1715, 1640, 1620 cm^{-1}; nmr (δ, $CDCl_3$) 1.76 (3H, s, CH_3C=), 2.5 (4H, m, $-CH_2CH_2-$), 2.5 (3H, s, CH_3CO-), 3.25 (2H, m, $ArCH_2C$=), 5.32 (1H, m, –CH=), 6.36 (1H, d, $J = 9$ Hz, ArH), 7.44 (1H, d, $J = 9$ Hz, ArH); MS (m/e) 278 (M^+, 24%), 252 (30%), 205 (26%), 191 (61%), 179 (77%), 165 (100%), 147 (32%). (Found: C 64.76; H 6.41. $C_{15}H_{18}O_5$ requires C 64.74; H 6.52.)

With compound (**11**) as substrate in the same conditions, a residue (175 mg) was obtained which was chromatographed as described before and which gave 27 mg of (**11**). From other fractions containing the mixture (**1**) + (**15**), 60 mg, compound (**15**), 25 mg, was obtained by preparative tlc (eluent benzene/methanol 9/1) as oily compound: nmr (δ, $CDCl_3$) 1.76 (3H, d, $J = 1$ Hz, CH_3–C=), 2.47 (4H, m, $-CH_2CH_2-$), 2.52 (3H, s, CH_3CO-), 3.3 (2H, m, $ArCH_2-$), 5.45 (1H, m, –CH=), 6.4 (1H, d, $J = 2$ Hz, ArH), 7.42 (1H, s, ArH); MS (m/e) 278 (M^+, 23%), 252 (30%), 205 (26%), 191 (61%), 179 (77%), 165 (100%), 147 (32%).

5,7-Dimethoxy-4-methylphthalan (**13**)

The solution of 500 mg of 5,7-dimethoxy-4-methylphthalide in 100 ml of tetrahydrofuran containing 10 ml of boron trifluoride diethyl etherate was added dropwise to a suspension of 300 mg of $LiAlH_4$ in 20 ml of tetrahydrofuran, the temperature being kept at 0°C. After 1 hr at 0°C the mixture was refluxed for 12 hr then cooled and dild HCl was added. After extraction with diethyl ether, washing of extract with water, drying (Na_2SO_4), and evaporation under reduced pressure, the residue was dissolved in 15 ml of diethyl ether. The insoluble starting material was filtered (98 mg), and the soluble fraction was chromatographed on 15 g of silica-gel Merck 0.05–0.2 mm eluting with *n*-octane–ethyl acetate 9/1 (20 ml fractions). Fractions 4–10 contained 200 mg of (**13**), which had mp 121–122°C (ethyl acetate); nmr (δ, $CDCl_3$) 2.01 (3H, s, CH_3Ar), 3.82 and 3.84 (3H, s, CH_3O-), 5.06 and 5.08 (2H, s, $ArCH_2OCH_2-$), 6.36 (1H, s, ArH). (Found: C 67.99; H 7.37. $C_{11}H_{14}O_3$ requires: C 69.02; H 7.27.) Fractions 20–29 contained 2,3-dimethyl-4,6-dimethoxybenzyl alcohol, mp 118–119°C (ethyl acetate); nmr (δ, $CDCl_3$) 2.30 (6H, s, CH_3Ar), 3.84 (6H, s, CH_3O-), 4.73 (2H, s, $-CH_2OH$), 6.47 (1H, s, ArH). (Found: C 67.16; H 8.29. $C_{11}H_{16}O_3$ requires: C 67.32; H 8.22.)

5,7-Dihydroxy-4-methylphthalan (**12**)

The solution of 300 mg of 5,7-dimethoxy-4-methylphthalan (**13**) in 16 ml of CH_2Cl_2 containing 1.85 ml of BBr_3 was kept at room temperature for 24 hr. After addition of this solution to ice, a precipitate was collected (200 mg) and crystallized from ethyl acetate to give 80 mg of (**12**), mp 195°C (dp). The filtrate was extracted with ethyl acetate, and the organic extracts and the mother liquor from the crystallization of the precipitate were collected and evaporated under reduced pressure. The residue thus obtained was chromatographed on 15 g of silica-gel Merck 0.05–0.2 mm using ECA as eluent. A further 90 mg of (**12**) was thus obtained; nmr (δ, DMSO) 1.86 (3H, s, CH_3Ar), 4.86 (4H, s, $-CH_2OCH_2-$), 6.23 (1H, s, ArH). (Found: C 64.91; H 6.27. $C_9H_{10}O_3$ requires: C 65.05; H 6.07.)

Alkylation of 2,4-Dihydroxyacetophenone (**4**)

In a solution of 800 mg of 3,4-dihydroxyacetophenone (**4**) in 100 ml of dioxane containing 2.3 g of *t,t*-farnesyl bromide was suspended 2 g of Ag_2O. After 4 hr in the dark with stirring, the suspension was filtered, and the residue from the evaporation of the filtrate under reduced pressure was chromatographed on 140 g of silica-gel Merck 0.05–0.2 mm eluting with *n*-octane–ethyl acetate 85/15 (20 ml fractions). Fractions 25–34 contained 200 mg of 2,4-dihydroxy-5-farnesylacetophenone (**11**) (oily compound); nmr (δ, $CDCl_3$) 3.2–3.4 (2H, m, $ArCH_2-$), 4.8–5.5 (3H, m, $-CH=$), 6.33 (1H, singlet, ArH), 7.43 (1H, singlet, ArH); ν_{max}(nujol) 1637, 1595 cm^{-1}. (Found: C 77.35; H 9.14. $C_{23}H_{32}O_3$ requires: C 77.48; H 9.05.) Fractions 39–50 contained 150 mg of 2,4-dihydroxy-3-farnesylacetophenone, mp 85–86°C (methanol–water); nmr (δ, $CDCl_3$) 3.37–3.6 (2H, m, $ArCH_2-$), 4.9–5.45 (3H, m, $-CH=$), 6.37 (1H, d, $J = 9$ Hz, ArH), 7.5 (1H, d, $J = 9$ Hz, ArH); ν_{max}(nujol) 1627, 1595 cm^{-1}. (Found: C 77.35; H 9.14. $C_{23}H_{32}O_3$ requires: C 77.49; H 9.05.) Fractions 55–70 crystallized from ethyl acetate–*n*-octane yielded 501 mg of (**4**).

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