the best performances of this compound with these 2 viruses.

1-Propyl-MBB also delays or prevents the onset of cytopathic change in MK cells infected with Coxsackie B 5 and A 9 and ECHO 6, 11 and 28 viruses and in ERK cells infected with Coxsackie A 21 virus.

1-Alkyl-2-(α-methoxybenzyl)benzimidazoles (I; R=alkyl, R'=Me) were prepared by heating, under reflux for 10 h, the appropriate *N*-alkyl-*o*-phenylenediamine[3] (1 mole) and α-methoxyphenylacetic acid (1 mole) in 2 *M* hydrochloric acid (2.5 moles). The 1-butyl derivative separated as the hydrochloride on cooling the reaction mixture and, after crystallization from either 2 *M* hydrochloric acid or ethanolic ether with charcoal treatment, formed white prisms (35.5% yield; m.p. 171.5–173°). In other cases, the reaction mixture was made alkaline with 3 *M* potassium carbonate, the separated base then extracted with chloroform and the extract washed with saturated sodium bicarbonate solution, followed by water, and dried over anhydrous sodium sulphate. The red oil remaining after evaporation of the solvent was converted into the picrate. Crystallization from ethanolic ether after treatment with charcoal gave a pure picrate in each case. Each base could be regenerated by suspending the pure picrate (1 g) in water (200 ml) and dissolving by slowly adding ethanol (ca. 170 ml) with warming. Then the solution was twice treated with Dowex 1 anion exchange resin (ca. 3 g, in the chloride form) and filtered. After concentrating the colourless filtrate by evaporation, an equivalent volume of *M* sodium hydroxide was added and the base extracted with chloroform and dried over anhydrous sodium sulphate. Removal of the solvent left the 1-alkyl-2-(α-methoxybenzyl)benzimidazole as an oil, which slowly crystallized except in the case of the 1-ethyl derivative. Details of the compounds are given in Table II.

Table II. 1-Alkyl-2-(α-methoxybenzyl)benzimidazoles (I, R = alkyl, R'=Me) and analytical data for their picrates

| R = | Me | Et | Pr | Bu |
|---|---|---|---|---|
| Form | White prisms | Colourless oil[a] | White prisms | Colourless oil[b] |
| m.p (°C) | 74.5–76 | | 59–60.5 | |
| Yield (%) | 30.9 | 45.4 | 37.7 | 34.5 |
| Form[c] | yellow needles | yellow prisms | yellow prisms | yellow prisms |
| m.p (°C)[c] | 188–189 | 181–182 | 190–191 | 182–183 |
| Formula[c] | ø[d] | ø + $CH_2$ | ø + $C_2H_4$ | ø + $C_3H_6$ |
| C Required (%) | 54.9 | 55.7 | 56.6 | 57.3 |
| Found (%) | 54.8 | 56.0 | 56.8 | 57.0 |
| H Required (%) | 3.99 | 4.28 | 4.56 | 4.83 |
| Found (%) | 4.08 | 4.38 | 4.49 | 4.93 |
| N Required (%) | 14.6 | 14.1 | 13.7 | 13.4 |
| Found (%) | 14.5 | 14.4 | 13.7 | 13.0 |

[a] $n_D^{23} = 1.5850$; [b] $n_D^{16} = 1.5778$; [c] refers to the picrates; [d] ø = $C_{22}H_{19}N_5O_8$.

The high lipid solubility of these methoxy compounds could prove a valuable feature in any future application of their antiviral properties[5].

*Zusammenfassung.* 1-Alkyl-2-(α-methoxybenzyl)-benzimidazole hemmt die Vermehrung des Poliovirus der Arten 1, 2 und 3, wobei das 1-Propyl-Derivat die grösste Wirkung hat.

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## 6-Methoxymellein as a Phytoalexin

3-Methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (I), often referred to as 6-methoxymellein, was isolated by Sondheimer[1] from carrots which had developed a bitter taste during storage. In a series of subsequent investigations[2–5], Kuć and his collaborators suggested that this fungitoxic compound is produced by alteration of the metabolism of the carrot root tissue induced by infection by one of several fungi and is a factor in the disease resistance mechanism of the carrot. This proposal has been elaborated in a recent review[6], and in another review[7] 6-methoxymellein has been classed as a 'phytoalexin'.

In a previous investigation (with Dr. J. Levi, 1964) we examined the growth of *Ceratocystis fimbriata* Ell. and Halst. (the fungus which was later reported by Kuć[5] to induce the greatest production of 6-methoxymellein in carrot slices) in submerged culture on synthetic media and obtained evidence for the production of phenolic metabolites related to those observed by us in *Aspergillus terreus* Thom.[8]. Since the report of Aue et al.[9] of the isolation of (I) from submerged culture of *Sporormia bipartis* Cain we have repeated our investigations, and the recent report of McGahren and Mitscher[10] of the isolation of (I) from another *Sporormia* sp. prompts us to record our results.

*C. fimbriata* Ell. and Halst. was grown in a standard corn-steep liquor medium[11] in shake flasks (60 ml medium)

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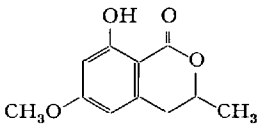
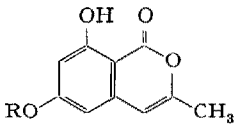
[10] W. J. McGahren and L. A. Mitscher, J. org. Chem. *33*, 1577 (1968).
[11] R. F. Curtis, P. C. Harries, C. H. Hassall and J. D. Levi, Biochem. J. *90*, 43 (1964).

for 11 days at 25 °C. The whole broth (ca. 7 l medium) was extracted with ether and after evaporation of the ether the residue was purified by preparative thin-layer chromatography to give the isocoumarin (II) (18 mg) m.p. 129°, identical in all respects with synthetic material[12] as the major phenolic metabolite. We were unable to isolate the dihydroisocoumarin (I) but several other closely related compounds were present. One of these has been identified (by thin-layer chromatography) as the dihydroxyisocoumarin (III)[13]; others are being investigated.

The production of compounds such as (II) and (III) so closely related to (I) in a synthetic medium must throw doubt on the real origin of 6-methoxymellein in carrot infected with *C. fimbriata*, and the status of (I) as a 'phytoalexin'. These results clearly support the reservations implied by AUE et al.[9] and MCGAHREN and MITSCHER[10] and suggest that some other phytoalexins might warrant further investigation[14].



(I)



(II; R = $CH_3$)
(III; R = H)

*Résumé.* Le dihydroisocoumarin (I) était considéré comme un «phytoalexin» produit par des carottes infectées de *Ceratocystis fimbriata*. Mais l'isocoumarin (II), de structure apparentée ayant été dégagé d'une culture de ce champignon sur bouillon synthétique, on peut douter que (I) tire son origine des carottes.

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[14] Acknowledgment. I am grateful to Prof. C. H. HASSALL for laboratory facilities, to Mr. M. J. HALL for advice and Misses J. MOLLETT and S. DARK for assistance.

## Ethyl 6,7-*bis*(Cyclopropylmethoxy)-4-hydroxy-3-quinolinecarboxylate, a Potent Anticoccidial Agent

Alkyl 6,7-dialkoxy-4-hydroxy-3-quinolinecarboxylates have been shown to exhibit a good order of anticoccidial activity in the chicken[1].

Our interest in substituted 4-hydroxyquinoline-3-carboxylates, extending back to 1962, prompted us to enlarge upon some earlier work. This endeavor yielded ethyl 6,7-*bis*(cyclopropylmethoxy)-4-hydroxyquinoline-3-carboxylate (Su-18,137), a substance showing outstanding activity against a number of species of coccidia.

The synthesis of Su-18,137 is outlined in the Figure.

Catechol was alkylated with chloro- or bromo-methylcyclopropane in dimethylformamide solution containing 2 equivalents of sodium hydride (oil dispersion) to give the corresponding diether (I), $b.p._{13}$ 162°C. Anal. Calcd. for $C_{14}H_{18}O_2$: C, 77.02; H, 8.31. Found: C, 76.78; H, 8.40.

Treatment of (I) with aqueous nitric acid gave the 4-nitro derivative (II), m.p. 80.5–81.5°. Anal. Calcd. for $C_{14}H_{17}NO_4$: C, 63.86; H, 6.51; N, 5.32. Found: C, 63.55; H, 6.42; N, 5.07. This substance was identical with that prepared from (III).

Catalytic hydrogenation of II in ethanolic solution in the presence of platinum oxide gave IV, which was converted to V without isolation by refluxing with 1 mole of diethyl ethoxymethylene malonate, m.p. 66–67°. Anal. Calcd. for $C_{22}H_{29}NO_6$: C, 65.49; H, 7.24; N, 3.47. Found: C, 65.90; H, 7.34; N, 3.69. Cyclization of V to VI occurred on refluxing in Dowtherm A® for 10–15 min. Anal. Calcd. for $C_{20}H_{23}NO_5$: C, 67.21; H, 6.49; N, 3.92. Found: C, 66.51, 66.50; H, 6.38, 6.34; N, 3.75. All structures were confirmed by means of NMR- and IR-analyses.

*Biological results.* Su-18,137 was mixed into chick starter feed and administered therein to 7-day-old white Leghorn chicks which were inoculated with coccidia 24 h later by intubation of sporulated oocysts into the crop. The medicated chicks were found to exhibit survival rates in this infection which caused high mortality in untreated birds. The results of 4 tests involving 3 different strains of *Eimeria tenella* are shown in Table I. Complete protection was obtained at dose levels as low as 0.0008%.

Su-18,137 was then submitted to a more rigorous test of activity. Infections with 5 species of mixed coccidia

Table I. Activity of SU-18,137 against *Eimeria tenella* in 7-day-old chicks infected with approximately 150,000 oocysts 24 h after initiation of medication in the feed (continued for 8 days)

| Test No. | *E. tenella* strain | Dose level (% in feed) | No. chicks started | No. chicks surviving on day 10 | % survival |
|---|---|---|---|---|---|
| 1 | 'N' | 0.0085 | 11 | 11 | 100 |
| | | 0.00425 | 10 | 9 | 90 |
| | | 0 (controls) | 18 | 3 | 23 |
| 2 | 'N' | 0.0032 | 10 | 10 | 100 |
| | | 0 (controls) | 20 | 0 | 0 |
| 3 | 'P' | 0.0008 | 10 | 10 | 100 |
| | | 0 (controls) | 9 | 4 | 44 |
| 4 | 'S' | 0.0008 | 8 | 8 | 100 |
| | | 0 (controls) | 10 | 3 | 30 |

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