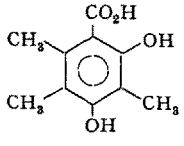
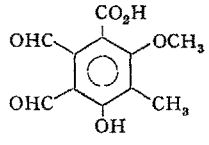
8.04, 7.92 and 7.65 $\tau$ (3 × Ar-C$H_3$) and − 0.36 $\tau$ ($CO_2H$, exch.), and a diacetoxymethyl ester, m.p. 123–124°; *Anal.* ($C_{15}H_{18}O_6$): C, 60.80; H, 5.73; $\nu_{max}^{KBr}$ 1735 cm$^{-1}$ (Ar-$CO_2CH_3$). The carbonyl shift upon acetylation suggested a salicylic acid[1]. In agreement with this, the substance readily decarboxylated upon heating or treatment with base to give the known 1,3-dihydroxy-2,4,5-trimethylbenzene, m.p. 152–153° (lit.[2], m.p. 156°) characterized also by mass spectrum, nmr [3.78 $\tau$ = Ar-$H$], analysis and conversion to its crystalline diacetate, m.p. 74–75°; *Anal.* ($C_{13}H_{16}O_4$): C, 66.06; H, 6.69. More vigorous alkaline treatment gave, inter alia, the known 2-hydroxy-3,5,6-trimethylbenzoquinone, m.p. and mixture m.p. 95.5–96°. The spectral properties of the 2 samples were also identical[3]. These findings are sufficient to establish the structure of the metabolite as I. The structural similarity of I and cyclopaldic acid[4] (II) is striking. These mold metabolites are highly substituted benzene derivatives and would seem to be related to 6-methylsalicylic acid by simple biosynthetic operations[5].



(I)



(II)

*Zusammenfassung.* Aus Kulturen *Mortierella ramanniana* var. *angulispora* (Naumov) Linnemann wurde 2,4-dihydroxy-3,5,6-trimethylbenzoesäure isoliert. Auf Grund seiner chemischen und physikalischen Eigenschaften wird ihm die Strukturformel I erteilt.

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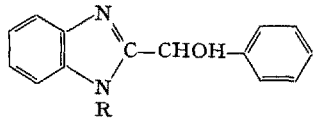
*Lederle Laboratories, A Division of American Cyanamid Company, Pearl River (New York 10965, USA), 12th May 1967.*

[1] L. J. BELLAMY, *The Infra-red Spectra of Complex Molecules* (John Wiley & Sons, Inc., New York 1958), p. 168.
[2] A. KRAUS, Mh. Chem. *12*, 203 (1891).
[3] W. FLAIG, J. C. SALFELD and E. BAUME, Justus Liebigs Annln Chem. *618*, 117 (1958). We thank Professor Dr. W. FLAIG for the gift of an authentic specimen.
[4] A. J. BIRCH and E. PRIDE, J. chem. Soc. *1962*, 370.
[5] Acknowledgments: we thank our colleagues Dr. P. SHU and associates for large-scale fermentations and the Organic Chemical Research Section of these Laboratories for the microanalytical and spectral data.

## New 1,2-Disubstituted Benzimidazoles with High Inhibiting Effects on Poliovirus Replication

Certain 1-substituted derivatives of 2-(α-hydroxybenzyl)-benzimidazole (I) are very active at inhibiting poliovirus multiplication[1–3].



(I)

Two new highly active derivatives (I; R = Ph) and (I; R = $-CH_2CHMe_2$) have been prepared[4], the phenyl derivative (I; R = Ph) being effective at lower concentrations than any other member of this series. Determination of toxicities and activities of these 2 compounds is complicated by the relatively low water solubilities of both the free bases and their hydrochlorides. However, dispersion of progressively larger quantities in nutrient medium, already saturated with compound, produces larger biological effects. It is probable that, during the course of experiments, undissolved compound slowly goes into solution to preserve equilibrium at the same time as dissolved compound undergoes change within the cells. 'Concentrations' of the 1-phenyl and 1-β-methylpropyl derivatives quoted in this report are purely nominal and given by the number of micromoles of compound dispersed per litre of medium. Both antiviral activities and toxicities, if based on the amounts of the 2 compounds actually in solution, might be greater than the present data suggest. A spectrofluorimetric assay has been developed for HBB (I; R = H) and its 1-alkyl derivatives (I; R = Alkyl) and this method might help to give information on the actual concentrations of these 2 compounds[5]. It should be noted, however, that quenching of fluorescence occurs on interaction between compounds of type (I) and nucleic acids[5] and this could confuse the exact assessment of concentration of 'free and bound' inhibitor.

Table I summarizes the results of a typical set of experiments comparing the protective actions of the 1-phenyl and 1-β-methylpropyl derivatives, at half their maximum tolerated concentrations, with the protection offered to tissue culture cells by HBB (I; R = H)[1–3]. Experiments were carried out with *ERK* cells growing in a nutrient medium on the glass surface of test tubes that were slowly rotated at 37 °C. Maximum tolerated concentrations (listed in Table II) were taken as the highest concentrations of the compounds producing no microscopically discernable damage to growing cells over a period of 4$^1/_2$ days. There was no microscopically visible change to cells grown continuously for 18 days in medium containing half maximum tolerated concentrations of either the 1-phenyl or the 1-β-methylpropyl compound. However, the rate of cell multiplication was less than in control tubes or in tubes containing either the propyl derivative PHBB (I; R = Pr) (40 μ*M*) or the parent compound HBB (I; R = H) (105 μ*M*).

Both 1-phenyl and 1-β-methylpropyl derivatives can considerably delay the onset of cytopathic effects and

[1] D. G. O'SULLIVAN and A. K. WALLIS, Nature *198*, 1270 (1963).
[2] D. G. O'SULLIVAN, D. Pantic and A. K. WALLIS, Nature *203*, 433 (1964).
[3] D. G. O'SULLIVAN, D. PANTIC and A. K. WALLIS, Nature *205*, 262 (1965).
[4] D. G. O'SULLIVAN, *Viruses and the Chemotherapy of Viral Diseases* (Royal Institute of Chemistry, Lecture Series 1965, No. 2), p. 33.
[5] D. G. O'SULLIVAN and A. K. WALLIS, unpublished work.

the 1-phenyl derivative is capable of completely protecting the cells against all 3 virus types at moderately high initial concentrations (Table I).

Equal dilutions of each virus type in medium alone and in medium containing either the 1-phenyl derivative (70 $\mu M$) or the 1-$\beta$-methylpropyl derivative (50 $\mu M$) were kept at 37 °C for 12 h. Assays of comparative virus concentrations showed, for each virus type, no significant difference between the virus concentrations in the tubes with or without test-compounds. Consequently the compounds have no direct inactivating effect on these viruses in the absence of cells.

Concentrations giving 75% inhibition of virus multiplication (virus inhibiting concentrations) for some very active 1-alkyl or 1-aryl derivatives are listed in Table II[4]. Activities of these 1-substituted compounds are greatest for the type 2 virus and least for the type 3 virus. Considering each virus type separately, inhibiting activities relative to the activity of HBB (I; R = H) and relative selectivities (that allow for the different toxicities) are given in Table III. Values of the activities and selectivities increase as the side-chain at position 1 lengthens, these functions reaching their maxima where the length is in the neighbourhood of 5.3 Å (1-phenyl derivative), and then they decline in value with further elongation of the side-chain (Table III). This trend is less regular with the type 3 virus than with the types 1 and 2 viruses (Table III). Other results, not included in Table III, follow the same trend. Thus the activities of the 1-methyl and 1-ethyl derivatives (chain lengths, 2.1 and 3.4 respectively) lie between those of HBB and PHBB (I; R = Pr)[1]. The 1-pentyl derivative (length, 7.1) is

Table I. Protection given by 1-phenyl-HBB (I; R = Ph), 1-$\beta$-methylpropyl-HBB (I; R = $-CH_2CHMe_2$) and HBB (I; R = H) to *ERK* cells infected with types 1, 2 and 3 poliovirus

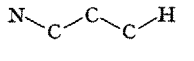
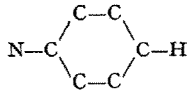
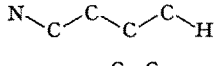
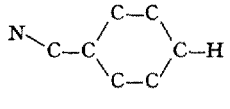
| Virus Type[b] | Mean delay (days) between infection with virus and degeneration of half the cell population[a] | | | | |
|---|---|---|---|---|---|
| | Dilution factor[c] | Infected controls[d] | 1-phenyl-HBB (70 $\mu M$) | 1-$\beta$-methyl-propyl-HBB (50 $\mu M$) | HBB (100 $\mu M$) |
| 1 | 10 | 1.0 | 4.5 | 1.0[e] | 1.0 |
| | 100 | 1.75 | 7.25 | 6.0 | 2.0 |
| | 1000 | 2.0 | 7.25 | 7.25 | 2.25 |
| 2 | 10 | 1.0 | 6.5 | 4.25 | 2.75 |
| | 100 | 1.5 | 7.25 | 6.0 | 3.75 |
| | 1000 | 2.0 | 7.25 | 7.25 | 4.5 |
| 3 | 10 | 0.75 | 3.75 | 1.5 | 1.0 |
| | 100 | 1.25 | 6.0 | 2.5 | 1.5 |
| | 1000 | 1.75 | 7.25 | 4.25 | 2.0 |

[a] Times are quoted to the nearest quarter of a day. [b] Virus strains used for the experiments in this paper: *L Sc, 2ab* (type 1); *P 712, Ch 2ab* (type 2) and *Leon 12ab* (type 3). [c] Approximate initial virus concentration (before dilution): $10^{7.2}$ $TCD_{50}$ U/ml (types 1 and 2 virus) and $10^{7.5}$ $TCD_{50}$ U/ml (type 3 virus). [d] Control tubes containing cells growing in the absence of both virus and protective agents lasted 7.25 days before half the cells had degenerated. [e] This low figure might be due to toxic contamination of these tubes.

Table II. Maximum tolerated concentrations (MTC) and the concentrations of 1-substituted 2-(α-hydroxybenzyl)-benzimidazoles (I) giving 75% inhibition of poliovirus multiplication in *ERK* cells

| Substituent R | MTC ($\mu M$) | Micromolarities giving 75% inhibition of poliovirus multiplication | | |
|---|---|---|---|---|
| | | Type 1 | Type 2 | Type 3 |
| H | 210 | 160 | 35 | 160 |
| $\beta$-Methylpropyl | 100 | 13 | 10 | 25 |
| Propyl | 80 | 9 | 7.5 | 22.5 |
| Phenyl | 140 | 6 | 5 | 15 |
| Butyl | 60 | 10 | 5.5 | 25 |
| Benzyl | 100 | 20 | 12.5 | 25 |

Table III. Length of side-chain ($L$)[a], relative activity ($A$)[b] and relative selectivity ($S$)[c] of 1-substituted 2-(α-hydroxybenzyl)benzimidazoles (I) (in relation to the inhibition of poliovirus multiplication in *ERK* cells)

| Substituent R | Chain of atoms | $L$ (Å) | Type 1 virus | | Type 2 virus | | Type 3 virus | |
|---|---|---|---|---|---|---|---|---|
| | | | $A$ | $S$ | $A$ | $S$ | $A$ | $S$ |
| H | N–H | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| $\beta$-Methylpropyl |  | 4.5 | 12.3 | 5.9 | 3.5 | 1.7 | 6.4 | 3.0 |
| Propyl | | 4.6 | 17.8 | 6.8 | 4.7 | 1.8 | 7.1 | 2.7 |
| Phenyl |  | 5.3 | 26.7 | 17.8 | 7.0 | 4.7 | 10.7 | 7.1 |
| Butyl |  | 5.9 | 16.0 | 4.6 | 6.4 | 1.8 | 6.4 | 1.8 |
| Benzyl |  | 6.1 | 8.0 | 3.8 | 2.8 | 1.3 | 6.4 | 3.0 |

[a] $L$ is the distance from centre of cyclic nitrogen atom to centre of terminal hydrogen atom in a planar all-*trans* arrangement as indicated in formulae in the preceding column. [b] $A$ is the concentration of HBB giving 75% inhibition of viral multiplication divided by the corresponding concentration for the other compound. [c] $S$ equals $A$ × maximum tolerated concentration of compound divided by maximum tolerated concentration for HBB.

notably less active than the butyl derivative[2]. Although not strictly comparable, the 1-β-methoxyethyl derivative (chain length 5.7) has high relative selectivity[3] and in this respect follows roughly the behaviour of the 1-alkyl and 1-aryl derivatives.

This relation between activities and chain lengths suggests that one property of the chemical structure contributing to its activity might increase and another might diminish, with the length of the carbon side-chain at position 1, in such a way that activity could be a maximum at the optimum chain length. Lipid and water solubilities might be 2 such properties and a suitable lipid to water partition coefficient may be of importance.

*1-Phenyl-2-(α-hydroxybenzyl)benzimidazole* was obtained by fusing an equimolar mixture of *o*-aminodiphenylamine and mandelic acid at 140° for $3^1/_2$ h. The glassy solid so obtained was extracted by hot *M* hydrochloric acid and the extract treated with charcoal and filtered. The cooled filtrate was made alkaline with 3*M* potassium carbonate and the precipitate collected and crystallized from aqueous methanol (with charcoal treatment) to give the *disubstituted benzimidazole* (36% yield) as white prisms, m.p. 184° (Anal.-Found: C, 80.0; H, 5.01; N, 9.5. $C_{20}H_{16}N_2O$ requires C, 80.0; H, 5.33; N, 9.3%). The *hydrochloride* crystallized from 2*M* hydrochloric acid as off-white prisms, m.p. 139.5–141°.

*1-β-Methylpropyl-2-(α-hydroxybenzyl)benzimidazole* was prepared from *o*-chloronitrobenzene and β-methylpropylamine via *N*-β-methylpropyl-*o*-nitroaniline and the corresponding diamine by the method previously described[1,2]. The *benzimidazole* was obtained, in 35% yield from the *o*-nitroaniline (32.5% overall from *o*-chloronitrobenzene), as white prisms from aqueous methanol after charcoal treatment, m.p. 174° (Anal.-Found: C, 77.0; H, 7.06; N, 10.2. $C_{18}H_{20}N_2O$ requires C, 77.1; H, 7.14; N, 10.0%). The *hydrochloride* crystallized from ethanolic ether as colourless needles, m.p. 196–7°.

Possessing activities of a high order, the compounds are promising as antiviral agents in spite of their relatively low solubilities in water[6].

*Zusammenfassung.* 1-Phenyl- und 1-β-Methylpropyl-2-(α-oxy-benzyl)-benzimidazol setzen die Vermehrung des Poliovirus der Arten 1, 2 und 3 in der Gewebekulturzelle stark herab. Die Hemmwirkungen und die Beeinflussung der Toxizitätsverhältnisse der 1-Phenyl-Derivate sind alle grösser, als die der früher untersuchten Benzimidazolene.

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*10th April 1967.*

[6] The research is supported by the National Fund for Research into Poliomyelitis and other Crippling Diseases.

## The Effect of Ammonium Ion upon Ribonucleic Acid Polymerase and Guanidine Triphosphatase Activity in Isolated Bovine Endometrial Nuclei

Administration of oestrogen to ovariectomized rats results in an enhanced rate of nucleoside triphosphate incorporation into ribonucleic acid (RNA) by nuclei prepared from uterine tissue.

GORSKI[1] noted that the uterine RNA polymerase activity was increased and the difference between oestrogen-treated and control nuclei was lost when concentrations of high ionic strength were present in the medium. Since TATA and WIDNELL[2] proposed the existence of 2 RNA polymerases in thyroid nuclei, one of which was $Mg^{++}$ and the other $Mn^{++}/NH_4$ dependent, the effect of ammonium ions upon uterine RNA polymerase was examined.

Nuclei isolated from cow endometrium were used. This offers an advantage because in contrast to nuclear preparations from total rat uterus or myometrium of cows, nuclei prepared from bovine endometrium appear microscopically to be devoid of myofibrils.

Cow uteri were supplied by a commercial source. The organs were immediately chilled and were used within 3–4 h after slaughter. The endometrium was collected by scraping. The stage of the ovarian cycle in cows was ascertained by the fern test of the cervical mucous, confirmed by gross and microscopic inspection of the ovaries.

Minced tissues were homogenized with a Lourdes Omni Mix at 5000 rpm in a medium containing 0.32*M* sucrose, 0.01*M* *Tris* HCl (pH 7.8) and 3 m*M* $MgCl_2$. The crude nuclear fraction was separated by centrifugation at 800 *g* and resuspended in 2.4*M* sucrose, 0.01*M* *Tris* HCl (pH 7.8) and 1 m*M* $MgCl_2$. This homogenate was spun at 25,000 *g* for 90 min at − 2 °C, and the pellet was suspended and washed twice in 0.25*M* sucrose, 0.01*M* *Tris* HCl (pH 7.8) and 4 m*M* $MgCl_2$.

RNA polymerase activity was assayed by measuring the conversion of $^{14}$C-nucleoside triphosphates into acid-insoluble material[3] under conditions that gave a linear rate of isotope incorporation.

Reactions to determine RNA polymerase activity were carried out in a final volume of 0.5 ml. The components were, unless stated otherwise: 0.2 ml nuclear suspension (100–400 μg DNA), 2.5 μ*M* $MgCl_2$, 0.5 μ*M* $MnCl_2$, 3 μ*N* NaF, 100 μ*M* *Tris* HCl (pH 7.8), 35 μ*M* KCl, 0.3*M* $(NH_4)_2SO_4$, 35 m*M* dithiotreitol, 50 μ*M* uridine triphosphate (UTP), guanosine triphosphate (GTP), cytidine triphosphate (CTP) and adenosine triphosphate (ATP), one of which was replaced by either 0.1 μc of $^{14}$C-UTP (S.A. 145 mc/m*M*) or 0.1 μc of $^{14}$C-GTP (S.A. 25 mc/m*M*) (Schwarz Bioresearch Laboratories. Orangeburg, New York, USA).

The nuclei were pre-incubated for 15 min at 37 °C prior to addition of radioactive isotope in order to exhaust the endogenous pool of nucleotides. Preliminary experiments had shown that the RNA polymerase activity was dependent upon the presence of all 4 nucleoside triphosphates. The reactions were terminated by addition of ice-

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[2] J. R. TATA and C. C. WIDNELL, Biochem. J. *98*, 604 (1966).
[3] W. C. SCHNEIDER, J. biol. Chem. *161*, 293 (1954).