lasted 90–150 min. As well as the effects mentioned, the s.c. injection of 0.5–1 μg/kg of caerulein caused a large output of pepsin.

Because of the close structural similarity between the *H. caerulea* peptide and gastrin, the effect of caerulein on gastric secretion of the dog was not unexpected. On a weight basis, caerulein was approximately 3 times as active as human gastrin-I[4].

In the perfused stomach preparation of the rat, caerulein produced a conspicuous increase in the total acid output measured over 20 min periods. The threshold dose was 15–25 ng/kg by the i.v. route and approximately 5 μg/kg by the s.c. route. For i.v. doses ranging between 20 and 200 ng/kg there was a good dose/effect relationship.

If, during the i.v. infusion of histamine at a rate itself ineffective or poorly effective on acid flow, caerulein is injected i.v., a conspicuous potentiation of the magnitude and an even greater potentiation of the duration of the secretory response to the polypeptide were observed. The histamine liberator compound 48/80 strongly reduced acid flow whilst aminoguanidine, an inhibitor of diamine-oxidase, increased it markedly.

On a weight basis, caerulein was 10–50 times as potent as human gastrin-I and 15–20 times as potent as carbachol.

Similar results were obtained when the rat stomach with ligated pylorus was used.

Preliminary experiments revealed that caerulein conspicuously increased the active transport of chloride by the isolated gastric mucosa of *Rana esculenta*. The threshold concentration was of the order of 0.003–0.01 ng/ml. On a weight basis, human gastrin-I was at least 300–1000 times less active (PESENTE et al., to be published).

(b) *Pancreatic secretion.* The administration of caerulein to anaesthetized dogs with the main pancreatic duct cannulated resulted, in each case, in a prompt increase in the volume of pancreatic juice. Unlike the juice produced by secretin but like that produced by pancreozymin, the juice produced by caerulein was rich in enzymes (amylase) and dry residue. The threshold dose was 3–6 ng/kg by rapid i.v. injection, 0.3–0.6 ng/kg/min by i.v. infusion and 100 ng/kg by the s.c. route. The magnitude of the response was directly related to the dose administered, and even at shock levels of blood pressure stimulation of pancreatic secretion could be observed.

If increase in volume flow only was considered, 1 μg caerulein was equiactive to 35–40 μg human gastrin-I, 1–3 Jorpes clinical units of secretin or 10–20 μg pure cholecystokinin-pancreozymin.

Results similar to those described above for anaesthetized dogs were obtained if anaesthetized cats were used.

(c) *Biliary secretion.* In anaesthetized dogs caerulein elicited a powerful contraction of the gall bladder and apparently increased the flow of hepatic bile.

An evident increase in the rate of biliary flow occurred in the anaesthetized rat after administration of caerulein. The threshold i.v. dose was 1 μg/kg. Single doses of 2–5 μg/kg caused, over an approximately 2 h period, a 20–30% increase in the volume of bile produced. The dry residue content and cholesterol content of caerulein-bile was as high, or higher than the dry residue and cholesterol content of control-bile. With repeated doses the effect was more intense and could be sustained for 4–6 h.

On a weight basis, human gastrin-I showed 2% of the activity of caerulein.

From the above data, it can be seen that the relatively small molecule of caerulein possesses an astonishingly versatile and powerful pharmacological activity. At the same time, it mimics many of the effects of bradykinin, gastrin and cholecystokinin-pancreozymin.

The study of one natural and several synthetic caerulein-like polypeptides is in progress.

Full reports and discussions of the experiments and results described in this paper will be published elsewhere.

Riassunto. La caeruleina, nonapeptide attivo della pelle di *Hyla caerulea*, possiede un insieme di potenti azioni farmacologiche sulla muscolatura liscia vasale ed extravasale e sulle secrezioni del tubo digerente. La caeruleina provoca una relativamente prolungata caduta della pressione del sangue nel cane e nel coniglio, contrae potentemente la muscolatura in situ dello stomaco, dell'intestino e soprattutto della cistifellea, stimola poderosamente la secrezione gastrica, la secrezione pancreatica e, in misura minore, la secrezione biliare. In queste sue multiformi azioni farmacologiche la caeruleina risulta più potente rispettivamente della bradichinina, della gastrina e della colecistochinina-pancreozimina.

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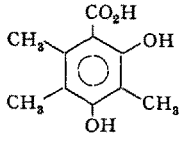
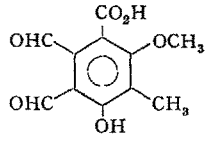
Isolation and Structure of 2,4-Dihydroxy-3,5,6-Trimethylbenzoic Acid from *Mortierella ramanniana*

We have isolated a highly substituted 6-methylsalicylic acid derivative from the fungus *Mortierella ramanniana* var. *angulispora* (Naumov) Linnemann and have determined its structure to be 2,4-dihydroxy-3,5,6-trimethylbenzoic acid (I). The fungus was grown with agitation and aeration for 5 days in a medium composed of glucose (3%), ammonium acetate (0.2%), sodium sulfate (0.1%), potassium acid phosphate (0.075%), potassium chloride (0.03%), magnesium acetate tetrahydrate (0.01%), ferric chloride hexahydrate (0.002%) and protein hydrolyzate (0.1%). The metabolite was recovered from the culture filtrate by solvent extraction and was purified by silica gel chromatography using benzene-ethyl acetate (4:1). Pure 2,4-dihydroxy-3,5,6-trimethylbenzoic acid (I), m.p. 192–193°, crystallizes from ethyl acetate-hexane. *Anal.* ($C_{10}H_{12}O_4$): C, 60.68; H, 6.28; O, 31.84; $M^+ = 196$ (mass spectrum); $[\alpha]_D^{25} \pm 0$; λ_{max}^{MeOH} (ε) 264 (11,950) and 310 nm (4410); alkali shifted the maxima to 256 (6940) and 302 nm (5000); ν_{max}^{KBr} 1620, 2860 ($ArCO_2H$) and 3500 cm^{-1} (ArOH); nmr (D_6-DMSO), 7.92 (2x $ArCH_3$), 7.60 ($ArCH_3$), 0.83 τ (broad 3×OH, exch.). The presence of 2 phenolic hydroxyls and a carboxyl was shown by facile formation of a diacetate, m.p. 151–155°; *Anal.* ($C_{14}H_{16}O_6$): C, 59.78; H, 6.13; ν_{max}^{KBr} 1690 ($ArCO_2H$), 1775, 1785 cm^{-1} ($ArOCOCH_3$), nmr ($CDCl_3$) 7.72 and 7.65 τ (2×$ArOCOCH_3$),

8.04, 7.92 and 7.65 τ (3 × Ar-CH_3) and − 0.36 τ (CO_2H, exch.), and a diacetoxymethyl ester, m.p. 123–124°; *Anal.* ($C_{15}H_{18}O_6$): C, 60.80; H, 5.73; ν_{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 τ = 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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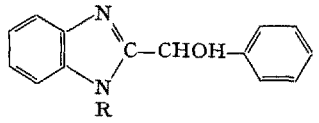
Lederle Laboratories, A Division of American Cyanamid Company, Pearl River (New York 10965, USA), 12th May 1967.

[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.