EXPERIENTIA

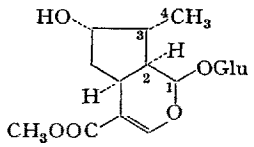
Vol. 24 – Fasc. 12 Pag. 1185–1296 15. 12. 1968

SPECIALIA

Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Ответственность за короткие сообщения несёт исключительно автор. – El responsable de los informes reducidos, está el autor.

Loganin from *Mytragyna parvifolia* Korth.

The importance of cyclopentane monoterpene skeleton in the biosynthesis of indole alkaloids has recently been emphasized[1], and loganin[2] (I) has been shown to be the precursor of these alkaloids by feeding (*o*-methyl (^{3}H))[3], (2-^{14}C)[4] and (4-^{14}C) loganins[5].



I

In the present communication we wish to report the isolation of loganin from the leaves of *Mytragyna parvifolia* Korth. (family Rubiaceae) which actively synthesize corynanthe[6] type of indole alkaloids and thus provide support, albeit circumstantial, for the intermediacy of loganin in the biosynthesis of indole alkaloids.

The polar non-alkaloidal fraction from 11 kg fresh leaves of *M. parvifolia* Korth. was separated by a combination of countercurrent distribution (100 transfers; solvent system; *n*-butanol-water) and column chromatography on silica gel (elution with ethyl acetate-ethanol mixtures) to give 300 mg loganin which was crystallized successively from absolute ethanol and methanol, m.p. and mixed m.p. with authentic sample, 221–222°. The identity was further confirmed by TLC, rotation, UV, IR, NMR and mass spectra[7] as well as by preparation of the penta-acetate, m.p. 140–141°.

This appears to be the first report of the isolation of loganin from a plant belonging to the Rubiaceae family, and thus indicates its close relationship with the plants of the Loganiaceae[8] family[9].

Zusammenfassung. Loganin, ein potentielles Schlüsselzwischenprodukt in der Biosynthese von Indolalkaloiden, wurde aus den Blättern von *Mytragyna parvifolia* Korth. isoliert.

D. S. Bhakuni and R. S. Kapil

Central Drug Research Institute, Lucknow (India), 24 July 1968.

[1] R. Thomas, Tetrahedron Lett. 544 (1961). – E. Wenkert, J. Am. chem. Soc. *84*, 98 (1962). – A. R. Battersby, Pure appl. Chem. *14*, 117 (1967).

[2] A. R. Battersby, R. S. Kapil and R. Southgate, Chem. Commun. 131 (1968). – S. Brechbühler-Bader, C. J. Coscia, P. Loew, Ch. v. Szczepanski and D. Arigoni, Chem. Commun. 136 (1968). – H. Inouye, T. Yoshida and S. Tobita, Tetrahedron Lett. 2945 (1968).

[3] A. R. Battersby, R. T. Brown, R. S. Kapil, J. A. Martin and A. O. Plunkett, Chem. Commun. 890 (1966).

[4] A. R. Battersby, R. S. Kapil, J. A. Martin and L. Mo, Chem. Commun. 133 (1968).

[5] P. Loew and D. Arigoni, Chem. Commun. 137 (1968).

[6] A. R. Battersby and R. S. Kapil, unpublished observations (1963).

[7] We thank Prof. A. R. Battersby for valuable suggestions and mass spectral data.

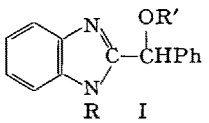
[8] J. Hutchinson, in *British Flowering Plants* (P. R. Gawthorn Ltd., London 1948), p. 374.

[9] Contribution No. 1291

The Inhibiting Actions on Poliovirus Multiplication of 1-Alkyl-2-(α-methoxybenzyl)benzimidazoles

2-(α-Hydroxybenzyl)benzimidazole (HBB: I; R=R'=H) and 2-(α-methoxybenzyl)benzimidazole (MBB: I; R=H, R' = Me), when tested simultaneously at half their respective maximum tolerated concentrations (MTC's)[1], exert broadly similar protective actions towards ERK cells infected with poliovirus[2]. Possession of similar activity by compounds with different solubility characteristics could be of practical value and it is noteworthy that the α-hydroxy group itself is not essential for marked activity in this series.

The protective actions of 1-alkyl derivatives of HBB (I; R=alkyl, R' = H) against the cytopathic effects of



I

[1] D. G. O'Sullivan, D. Pantic and A. K. Wallis, Experientia *23*, 704 (1967).

[2] D. G. O'Sullivan, D. Pantic and A. K. Wallis, Nature *205*, 262 (1965). – D. G. O'Sullivan and A. K. Wallis, J. chem. Soc. 2331 (1965).

polioviruses on ERK cells[3] and the inhibiting actions of these compounds on the multiplication of polioviruses (Table I) both increase with increase in alkyl-chain length to a maximum with the propyl or butyl derivative[4].

We have carried out studies with simple 1-alkyl derivatives of MBB (I; R = alkyl, R′ = Me) using type 1 (*L Sc, 2 ab*), type 2 (*P 712, Ch 2 ab*) and type 3 (*Leon 12 ab*) polioviruses in ERK cells. The hydrochloride of the 1-butyl derivative and the free bases of the other derivatives were used in our experiments. Dimethylsulphoxide was not employed to aid dispersion. As usual, broadly similar trends were shown with each of the 3 poliovirus types and activities were greater against the type 2 than against the types 1 and 3 viruses.

The toxicities towards ERK cells of MBB and its 1-methyl, 1-ethyl and 1-propyl derivatives are lower than those of HBB and its corresponding 1-alkyl derivatives. Maximum tolerated concentrations of 1-alkyl derivatives of MBB decline sharply to that of the 1-butyl derivative, which has the same MTC as 1-butyl-HBB (Table I). 1-Butyl-MBB influences the behaviour of ERK cells at concentrations well below its MTC. For example, the rate of metabolism of glucose is greatly reduced by the presence of only $^1/_4$ MTC of 1-butyl-MBB. Interference with normal cellular processes by sub-toxic concentrations of 1-butyl-MBB and the low solubility both of this compound and of its hydrochloride could account for its erratic behaviour in our tests.

Concentrations of compounds giving 75% inhibition of poliovirus multiplication after 16 h (VIC's)[4] and relative activities and relative selectivities[1] are listed in Table I.

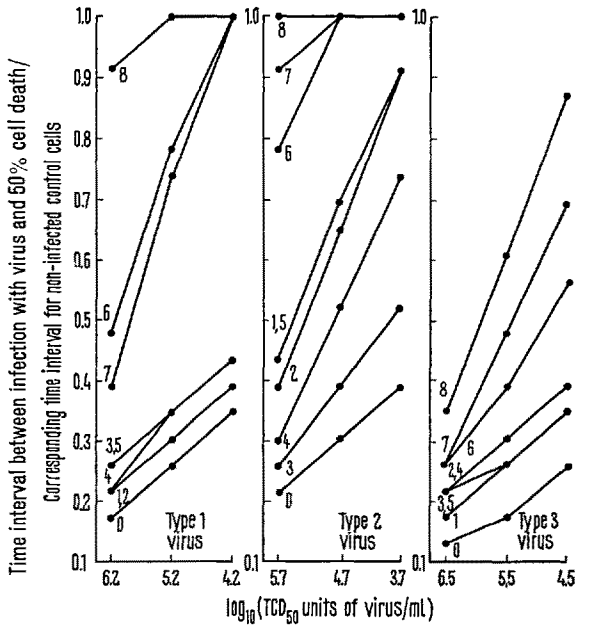
Table I. Maximum tolerated μmolarities (MTC), μmolarities giving 75% inhibition of poliovirus multiplication (VIC), relative activities (*A*)[1] and relative selectivities (*S*)[1] of HBB (I; R=R′=H), MBB (I; R=H, R′=Me) and their 1-alkyl derivatives

Virus type	Parent compound		Substituent R of virus inhibitor present				
			H	Me	Et	Pr	Bu
MTC	–	HBB	210	210	180	80	60
VIC	1		160	120	100	9	10
	2		35	35	30	7.5	5.5
	3		160	140	120	22.5	25
A	1		1	1.3	1.6	17.8	16.0
	2		1	1.0	1.2	4.7	6.4
	3		1	1.1	1.3	7.1	6.4
S	1		1	1.3	1.4	6.8	4.6
	2		1	1.0	1.0	1.8	1.8
	3		1	1.1	1.1	2.7	1.8
MTC	–	MBB	300	360	300	180	60
VIC	1		145	140	120	45	80
	2		40	50	35	20	35
	3		160	150	130	60	80
A	1		1.1	1.1	1.3	3.6	2.0
	2		0.9	0.7	1.0	1.8	1.0
	3		1.0	1.1	1.2	2.7	2.0
S	1		1.6	2.0	1.9	3.1	0.6
	2		1.3	1.2	1.4	1.5	0.3
	3		1.4	1.8	1.8	2.3	0.6

Quantities *A* and *S* (both relative to HBB for each virus) are defined in ref.[1].

1-Alkyl derivatives of MBB have lower relative activities than the corresponding derivatives of HBB, but a broadly similar general trend is present within the 2 series of compounds with relative activities and relative selectivities of 1-alkyl-MBB's rising to a maximum with the 1-propyl substituent. Different values for the VIC of 1-butyl-MBB were obtained in different determinations. Table I lists the lowest values obtained for this compound.

The Figure portrays the times of development of cytopathic change in ERK cells that were simultaneously infected with polioviruses and treated with $^1/_2$ MTC of compounds and also the corresponding times for infected control cultures. The most striking protection in the 1-alkyl-MBB series is given by the 1-propyl derivative. In the experiments with the type 2 and type 3 virus, the protective actions increase with alkyl chain length up to the propyl group. With the type 2 virus, there is a drop in going from the parent MBB to its 1-methyl derivative before the trend is followed. With the type 1 virus, MBB and its 1-methyl and 1-ethyl derivatives have similar effects. The behaviour of the 1-butyl derivative in different series of experiments is variable. All data obtained for each virus in the Figure were obtained from the one experiment. The protective action shown by 1-butyl-MBB against the type 2 virus is illustrative of its best performance with this virus, but the results portrayed with both the type 1 and type 3 viruses are well below



Protection given by $^1/_2$ MTC's of compounds to ERK cells infected with 3 dilutions of each poliovirus type. In the graphs, numbered lines join the 3 points for each compound as follows: line (0), controls with no test compound; (1), 1-butyl-MBB; (2), HBB; (3), 1-methyl-MBB; (4), 1-ethyl-MBB; (5), MBB; (6), 1-propyl-MBB; (7) 1-propyl-HBB (PHBB) (20 μ*M*); (8), PHBB (40 μ*M*). Half the non-infected control cells survived 5.75 days in all the 3 experiments portrayed. The presence of $^1/_2$ MTC of any of the test compounds made no difference to these survival times for non-infected control cells.

[3] D. G. O'SULLIVAN and A. K. WALLIS, Nature *198*, 1270 (1963).
[4] D. G. O'SULLIVAN, in *Viruses and the Chemotherapy of Viral Diseases* (Royal Institute of Chemistry, Lecture Series 1965, No. 2), p. 33.

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.

D. G. O'Sullivan, Danica Pantic and A. K. Wallis

Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, W.1. (England), 8 August 1968.

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

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)

[1] E. Sondheimer, J. Am. chem. Soc. *79*, 5036 (1957).
[2] P. Condon and J. Kuć, Phytopathology *50*, 267 (1960).
[3] P. Condon and J. Kuć, Phytopathology *52*, 182 (1962).
[4] P. Condon, J. Kuć and H. N. Draudt, Phytopathology *53*, 1244 (1963).
[5] B. A. Herndon, J. Kuć and E. B. Williams, Phytopathology *56*, 187 (1966).
[6] J. Kuć, A. Rev. Microbiol. *20*, 342 (1966).
[7] I. A. M. Cruikshank, A. Rev. Phytopath. *1*, 357 (1963).
[8] R. F. Curtis, P. C. Harries, C. H. Hassall, J. D. Levi and D. M. Phillips, J. chem. Soc. (C), 168 (1966).
[9] R. Aue, R. Mauli and H. P. Sigg, Experientia *22*, 575 (1966).
[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).