fore be related to fraction 1-V1 of KELLY[2], who found that their removal did not leave the blue fraction entirely pure. This may be explained by the presence of the purple component which is not eluted from an alumina column with aqueous organic solvents[5]. The absorption spectrum of the purple dye resembles those of the red-violet and blue-violet components and consequently this dye will also be recognized as a red impurity. In fact, the capillary test of HARTWELL and FIESER[1] detects preferentially this component which on paper chromatography with water as solvent moves in front of the other components with an Rf of 0.90. This dye may have been separated by KELLY[2] as fraction 1-V2 by passing an aqueous solution of trypan blue through a cellulose column. The purple dye has probably structure III because electrophoresis after reduction with sodium dithionite revealed *o*-tolidine in the direction of the cathode. This dye is active in producing reticulosis in the liver of rats and is presumably responsible for the carcinogenic activity of commercial trypan blue[5].

It may be concluded that the so-called red impurity of trypan blue consists of one or more components depending on the method which is used for detection or extraction. Aqueous organic solvents usually extract a mixture of I and II while water preferentially elutes a third impurity (presumably III), the absorption spectrum of which resembles the spectra of the other reddish components.

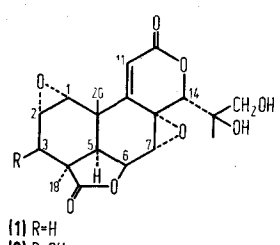
Zusammenfassung. Die sogenannte rote Verunreinigung von Trypanblau wurde als Gemisch von 3 Azofarbstoffen erkannt, voneinander getrennt und in ihrer Struktur aufgeklärt. Eine dieser Substanzen scheint für die kanzerogene Aktivität des kommerziellen Trypanblaus verantwortlich zu sein.

J. DIJKSTRA

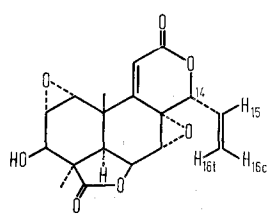
National Chemical Research Laboratory, P.O. Box 395, Pretoria (Republic of South Africa), 24 September 1971.

Plant Growth Inhibitory Lactones from *Podocarpus neriifolius:* Structure of Podolactone E

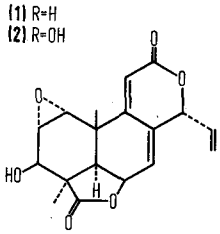
Earlier[1] we reported the bark of a *Podocarpus* species (c.f. *P. neriifolius* D. Don ex Lamb.) from Northern Queensland to contain two plant growth inhibitory compounds, podolactones A (**1**) and B (**2**). Further fractionation of the extract has afforded 4 other norditerpene lactones of the same type, podolactones C, D, E and the known lactone inumakilactone B (**3**)[2], identified by direct comparison with an authentic sample[3]. Podolactone E and inumakilactone B exhibit high activity as inhibitors of cell expansion in an assay system employing pea stem segments[4], inhibiting growth of hook and apical segments of pea stems at concentrations considerably lower than those required for the other podolactones and related compounds. The activities, expressed as the concentration necessary to limit growth of hook segments in 24 h to half that of the control, of podolactones A, B, E, inumakilactone B and abscisic (a convenient standard inhibitor) were respectively 60, 200, 6, 10 and 500×10^{-7} *M*. Podolactone E, which is shown to have the structure (**4**), is the most active inhibitory compound so far examined with this system.



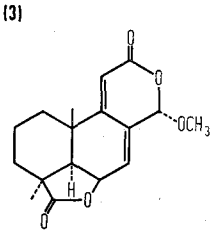
(1) R=H
(2) R=OH



(3)



(4)



(5)

Chemical shifts (δ) and coupling constants (Hz) of proton resonances[a]

a) From first-order analysis

	Podolactone E	Inumakilactone B[2]	Antibiotic LL-Z1271α[6]
1-H	3.69d	3.63d	
	(4.5)	(4.0)	
2-H	3.56dd	3.51dd	
	(4.5, 6.0)	(4.0, 5.6)	
3-H	4.69d	4.67d	
	(6.0)	(5.6)	
5-H	2.11d	2.16d	
	(5.5)	(5.2)	(5.3)
6-H	5.06m	5.10dd	
	(5.5, 4.0, 1.7)	(5.2, 1.2)	(5.3, 4.7, 1.0)
7-H	6.24m	3.95d	
	(4.0, 1.8, 2.0)	(1.2)	(4.7, 1.8, 2.0)
11-H	6.51d	6.78s	
	(1.8)		(1.8)
18-H_3	1.47s	1.41s	
20-H_3	1.52s	1.51s	

b) From computer analysis

	Podolactone E	Inumakilactone B
14-H	5.56	5.41
15-H	6.09	5.98
16c-H	5.48	5.42
16t-H	5.53	5.56
$J_{14,15}$	7.6	7.81
$J_{14,16^c}$	−0.6	−0.57
$J_{14,16^t}$	−0.7	−0.69
$J_{15,16^c}$	10.6	10.27
$J_{15,16^t}$	17.3	17.35
$J_{16^c,16^t}$	1.3	1.40

[a] In [$^2H_5$]pyridine.

[1] M. N. GALBRAITH, D. H. S. HORN, J. M. SASSE and D. ADAMSON, Chem. Commun. *1970*, 170.
[2] S. ITÔ, M. SUNAGAWA, M. KODAMA, H. HONMA and T. TAKAHASHI, Chem. Commun. *1971*, 91.
[3] We are grateful to Professor S. ITÔ, Tohoku University, for this sample.
[4] D. ADAMSON, V. H. K. LOW and H. ADAMSON, in *Biochemistry and Physiology of Plant Growth Substances* (Eds. F. WIGHTMAN and G. SETTERFIELD; Runge Press, Ottawa 1968), p. 505.

Podolactone E (**4**), $C_{18}H_{18}O_6$[5] (molecular-ion peak at *m/e* 330), m.p. 261–262°, shows hydroxyl absorption at 3500 cm^{-1} in its IR-spectrum and forms a monoacetate, $C_{20}H_{20}O_7$, m.p. 256–257°, $[\alpha]_D^{20}$ −30°. An IR-band at 1770 cm^{-1} indicates a γ-lactone group, while a band at 1720 cm^{-1} and UV-absorption at 257 nm (ε 14400) suggest the presence of a diene-lactone group similar to that found[6] in antibiotic LL-Z1271α (**5**). The NMR-spectrum of podolactone E (Table) bears an obvious similarity to that of inumakilactone B (**3**), while the differences in the spectra are accommodated by assignment of structure (**4**) to podolactone E. Thus the signal attributed to H-7 appears in the vinylic region of the spectrum (clearly seen in the spectrum of 15,16-dihydro-podolactone E acetate [see below] as a multiplet at δ 6.27), and exhibits couplings to H-6, H-11, and H-14, while H-6 shows homoallylic coupling to H-14. A vinyl side chain, as in inumakilactone B, was assigned to podolactone E on the basis of the close similarity of the appropriate regions of the NMR spectra of both compounds. This assignment was supported by hydrogenation of podolactone E acetate to the 15,16-dihydroderivative, $C_{20}H_{22}O_7$, m.p. 282° (dec.), λ_{max} 258 nm (ε 13800), which in its NMR-spectrum exhibited a 3 proton triplet (δ 1.05, J 7 Hz in pyridine) attributed to the C-16 methyl group.

The close agreement of the coupling constants found for the A-ring protons in the NMR-spectrum of (**4**) with the corresponding constants for (**3**), and for the B- and C-ring protons with those of (**5**), indicate that the stereochemistry depicted in (**4**) can be confidently assigned to podolactone E.

Podolactone E is very likely a biogenetic precursor of inumakilactone B, which can be formed by epoxidation of the 7,8-double bond: hydration of the side chain of the latter compound may then lead to inumakilactone A. The structural similarity of podolactone E and LL-Z1271α (**5**), which exhibits[6] antifungal activity, is of interest.

Résumé. Le Podolactone E, qui a la structure (**4**), empêche très activement la croissance des cellules végétales. C'est le plus actif des podolactones isolés jusqu'à présent.

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[5] Molecular formulae of all new compounds were established by microanalysis.

[6] G. A. ELLESTAD, R. H. EVANS, M. P. KUNSTMANN, J. E. LANCASTER and G. O. MORTON, J. Am. chem. Soc. *92*, 5483 (1970).

Anti-Hypercholesterolemic Effect of a Sulfur Containing Amino Acid, S-Methyl-L-Cysteine Sulfoxide, Isolated from Cabbage

S-methyl-L-cysteine sulfoxide (CH_3–S(=O)–CH_2–$CH(NH_2)$–COOH) (SMCS) is distributed abundantly in numbers of species of Cruciferae and Liliaceae plants[1–3]. For example according to the study of TSUNO[4] of our department, amounts of SMCS are 590 mg/100 g in cabbage, 650 mg/100 g in cauliflower or 60 mg/100 g in radish. The production of main Cruciferae plants in Japan represents some 7 million tons annually. Accordingly, annual production of SMCS and its analogues is estimated to be about 12,000 tons. Division of these amounts according to Japanese population results in over 300 mg of SMCS per person daily. This fact suggests that this amino acid from vegetable food may be one of the largest sources of sulfur-containing amino acids for Japanese; however, nutritional values of this amino acid are still obscure. The present report describes the anti-hypercholestrolemic effect of this amino acid.

Crystals of SMCS (m.p. 173°C) used in the experiment were isolated from cabbage and recrystallized by the method of TSUNO et al.[4], a modification of the method of MORRIS and THOMPSON[2].

Twenty-four male Wistar rats weighing about 100 g were separated into 4 groups. A synthetic diet was administered to each group and hydrogenated cocoanut oil was used as the source of fat. Composition of the basal diet is shown in Table I. Group 1 was given a basal diet, group 2 a basal diet supplemented with 1% cholesterol and 0.2% cholic acid, group 3 a basal diet supplemented with 0.25% SMCS, 1% cholesterol and 0.2% cholic acid. A diet which contained 0.5% SMCS, 1% cholesterol and 0.2% cholic acid was administered to the 4th group. A restricted diet (7–10 g) was prescribed every morning. Daily food intake amounts of each group was the same throughout the experimental period (average 8.6 g/day/rat). Dosage rates of SMCS in group 3 and group 4 were 182.2 mg and

Table I. Composition of the basal diet

	g/100 g diet
Casein	15.0
Sucrose	68.3
Hydrogenated cocoanut oil	10.0
Salt mixture[a]	4.0
Cellulose	2.0
Vitamin mixture[b]	0.5
Choline chloride	0.2

[a] The salt mixture contained: (% in the mixture) NaCl, 4.6; $Na_2HPO_4 \cdot H_2O$, 9.3; K_2HPO_4, 25.6; $CaH_4(PO_4)_2 \cdot H_2O$, 14.5; $Fe(C_6H_5O_7) \cdot 5H_2O$, 3.2; $Ca(C_3H_5O_3)_2\ 5H_2O$, 34.9; $MgSO_4$, 7.0; KI, 0.9. [b] 100 g of the vitamin mixture contained: (in mg) riboflavin, 150; thiamine, 100; nicotinic acid, 1000; pyridoxin, 100; cyanocobalamin, 1; pantothenic acid, 500; folic acid, 50; ascorbic acid, 3750; vitamin E, 100; vitamin A, 250,000 IU; vitamin D_2, 20,000 IU; and sucrose to 100 g.

[1] S. YURUGI, T. MATSUKAWA and M. TOGASHI, Pharm. Soc. Japan *74*, 1017 (1955).

[2] C. J. MORRIS and J. F. THOMPSON, J. Am. chem. Soc. *78*, 1605 (1956).

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