

LETTERS TO THE EDITOR

Calophyllolide, a complex coumarin anticoagulant from *Calophyllum inophyllum* Linn

SIR,—*Calophyllum inophyllum* Linn (Guttiferae) is an Indian medicinal plant commonly growing in the coastal regions of South India, Andaman islands, Burma and Ceylon. Ormancy, Potier, Buzas, and Lederer (1951) isolated a lactone, Calophyllolide from the defatted kernels of the nuts. Polonsky (1956) established its chemical structure as 3,4-dihydro-5-methoxy-2,2-dimethyl-6-(2-methylcrotonoyl)-10-phenyl-2*H*,8*H*-benzo[1,2-*b*:3,4-*b'*]dipyran-8-one.

The anticoagulant activity of calophyllolide, a coumarin derivative, was compared in 78 rabbits with dicoumarol, ethyl biscoumacetate and nicoumalone at the single dose levels of 50 mg./kg. by mouth for the hypoprothrombinaemic property, by determining the prothrombin time by Quick's (1935) one-stage method. The coagulation valency (the percentage prothrombin in plasma) in plasma from 35 normal Indian rabbits was established by the procedure of Montigel and Pulver (1952) in these laboratories for the evaluation of the results thus obtained. The technique used for determining the effect of calophyllolide on platelet clumping time was that of Mills, Nechles and Chu (1928) as modified by Sharp (1958). Whole blood coagulation time by Lee and White's method and bleeding time by Duke's (1910) technique were also studied and compared with dicoumarol. The results are summarised in Table I.

TABLE I
ANTICOAGULANT ACTIVITY OF CALOPHYLLOLIDE AND OTHER ANTICOAGULANTS
50 MG./KG. IN RABBITS

Property	No. of expts. with each drug	Calophyllolide	Dicoumarol	Ethyl biscoumacetate	Nicoumalone
Hypoprothrombinemic activity ..	12				
Coagulation valency, per cent ..		20*	10	50	35
Onset of action		24 hr.	24 hr.	24 hr.	24 hr.
Peak of action		48 hr.*	144 hr.	24 hr.	48 hr.
Duration of action		168 hr.	288 hr.	72 hr.	96 hr.
Platelet clumping time	5	35†	46†		
Whole blood coagulation time ..	5	11.2†	15.7†		
Bleeding time	5	18.35†	22.42†		

* Peak of hypoprothrombinemic action.

† Increase per cent, in time from normal.

It is evident from the results that the onset of hypoprothrombinaemic activity was achieved earlier with calophyllolide than with dicoumarol and nicoumalone, but more slowly than with ethyl biscoumacetate. Its duration of action is longer than ethyl biscoumacetate and nicoumalone but shorter than dicoumarol. It lowered the coagulation valency more than nicoumalone and ethyl biscoumacetate but slightly less than dicoumarol. The properties of calophyllolide, therefore, permit a relatively rapid onset of hypoprothrombinaemia like nicoumalone with less likelihood of the very rapid fluctuations of prothrombin time. In this sense, calophyllolide strikes a balance between the slow, long acting dicoumarol and the very fast, short acting ethyl biscoumacetate.

The whole blood coagulation, bleeding and platelet clumping times were also increased moderately like dicoumarol.

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Calophyllolide was given to five male rabbits for a period of eight weeks. A first dose of 50 mg./kg. brought down the coagulation valency to between 18 to 22 per cent after 48 hr. The subsequent doses of 30 mg./kg. on alternate days thereafter maintained the coagulation valency between 20 and 30 per cent. These rabbits were then killed after eight weeks and on macroscopic or microscopic examination of liver, kidney, spleen, skin and mucous membrane, revealed no evidence of haemorrhage. Further studies are in progress.

No fatalities were observed in rats when calophyllolide was administered orally in doses up to 1,750 mg./kg. Its intraperitoneal LD50 in rats was found to be 560 mg./kg.

This work was supported by financial assistance from The Council of Scientific and Industrial Research, New Delhi.

Our thanks are due to Professor T. R. Seshadri, F.R.S., Head of the Chemistry Department, University of Delhi, for supplying calophyllolide.

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June 1, 1962.

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Effect of Hydrocortisone on Capillary Permeability Changes Induced by *Echis carinatus* (Saw-scaled Viper) Venom in the Rat

SIR,—During recent years the corticosteroids have been reported to be very useful, alone and in combination with the specific antivenene, in the treatment of snake poisoning (Wig and Vaish, 1960; Gupta, Bhargava and Sharma, 1960; Benyajati, Keoplung and Srihibhadh, 1961; Arora, Wig and Somani, 1962). Since a widespread capillary endothelial damage, with the resultant haemorrhages in various viscera, in the skin, in the mucous membranes and the accompanying shock, determine to a large extent the ultimate outcome of viperine snake poisoning in man (Ahuja and Singh, 1956), we have examined the effect of hydrocortisone on the capillary permeability changes induced by the *Echis carinatus* (the saw-scaled viper, a common poisonous snake in India) venom in an attempt to elucidate the exact mechanism of protective action.

Azovan blue dye, 20 mg./kg., was administered intravenously in male albino rats, Haffkine strain, weighing 150-200 g., and the extent and intensity of blue discoloration produced by 20 µg. each of 5-hydroxytryptamine (5-HT), histamine and the *E. carinatus* venom (freeze-dried form, dissolved fresh before use) administered in 0.1 ml. distilled water intradermally in the previously depilated abdominal skin of the rat was observed. An interval of 15 min. was allowed for the dye to accumulate at the site of the drug administration when the rats were killed and the skin removed (see Parratt and West, 1957).