the vasa recta countercurrent system. The vasa recta of *Aplodontia*, beaver, and pig may not be able to trap urea, or the difference in their form may produce differences in the way in which the nephron handles urea.

Schmidt-Nielsen and O'Dell (*13*) have shown that in the kidneys of sheep conserving urea, the urea concentration rises most dramatically in the inner stripe of the outer zone, and these authors state that this zone comprises a very important part of the concentrating mechanism. In the rat (*14*) the outer zone of the medulla, as well as the papilla, has a high NaCl concentration, and water is not reabsorbed although it is reabsorbed in the inner zone. It is, therefore, only in the inner zone of the medulla that the gradient of increasing osmolarity toward the papilla is seen. Histochemical studies of the rat renal medulla reveal that the concentration of plasma proteins in the plexuses of the inner stripe of the outer zone and of the apex of the papilla is higher than that of the vasa recta linking the two regions (*15*), and show that the vasa recta traversing the inner stripe of the outer zone are fully developed retia with two-directional flow (*16*).

The presence of a special capillary plexus around the segments of the nephron which comprise the outer zone supports the concept that it is very active physiologically in those species showing zonation. The lack of such zonation in species showing no enhancement of urine concentration after a high protein diet suggests that a segment of the nephron may function differently in these species as compared to those showing pronounced zonation.

It would, therefore, seem that there are species differences in the form of the vasa recta and associated vessels of the mammalian renal medulla. These differences may have as much significance in the urine concentrating process as do the anatomical differences in the nephron of different mammalian species.

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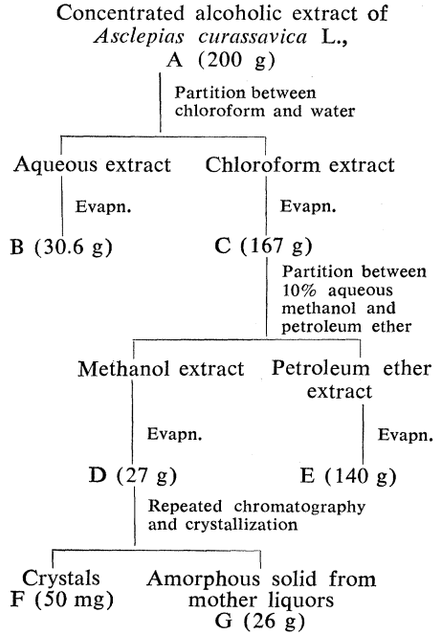
Calotropin, a Cytotoxic Principle Isolated from Asclepias curassavica L.

Abstract. *An alcoholic extract of* Asclepias curassavica *L., a plant widely used in folk medicine for treating cancer and warts, shows cytotoxic activity when tested in vitro against cells derived from human carcinoma of the nasopharynx. Systematic fractionation of the extract has led to isolation and characterization of calotropin as a cytotoxic principle. Calotropin is similar in structure to two cardiac glycosides recently shown to be responsible for the cytotoxicity of* Apocynum cannabinum *L.*

Asclepias curassavica L. (Asclepiadaceae), "cancerillo," and related species have been used for years to treat cancers, tumors, and warts in Costa Rica (*1*), Mexico (*2*), India (*3*), and elsewhere (*4*). During our search for tumor inhibitors from plant sources, alcoholic extracts of dried *A. curassavica* from Costa Rica (separated samples of roots, stems, and leaves) and from Mexico (a mixed sample of roots, stems, leaves, and flowers) (*5*) showed significant inhibitory activity when tested in vitro against cells derived from human carcinoma of the nasopharynx (*6, 7*). We report herein the fractionation of two active extracts and the isolation and characterization of a cytotoxic principle which is identified as calotropin.

Solvent partition of the alcoholic extract (*A* in Fig. 1) of the dried Mexican sample between water and chloroform resulted in a concentration of the activity in the chloroform phase (*C*). The dark gummy residue from the chloroform layer was de-fatted by partitioning between 10 percent aqueous methanol and petroleum ether (Skellysolve B), whereupon activity was concentrated in the aqueous methanol layer (*D*). Column chromatography of fraction *D* on silicic acid (using chloroform and chloroform-methanol as eluents) and analysis of the fractions by thin-layer chromatography and paper chromatography revealed at least seven components reactive to *m*-dinitrobenzene and sodium hydroxide (*8*). A compound of R_F 0.62 upon paper chromatography in the system chloroform-formamide was separated from the remainder of the material by further



Fraction	ED_{50} (*7*) (μg/ml)
A	1.3
B	9.8
C	1.9×10^{-1}
D	5.6×10^{-2}
E	5.1×10^{1}
F	3.7×10^{-3}
G	1.1

Fig. 1. Fractionation of a cytotoxic principle of *Asclepias curassavica* L., and cytotoxicity (*7*) of the fractions *A* through *G*.

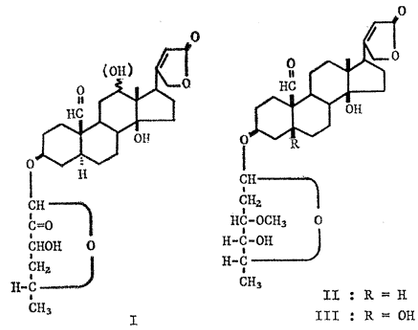


Fig. 2. Calotropin.

chromatography on silicic acid; the compound crystallized from methanol-ether as rosettes (*F*, 50 mg); melting point (mp), 195° to 202°C. The mother liquors from the crystallization of *F* were combined and chromatographed on silica gel plates with 13 percent methanol in chloroform. The band corresponding to calotropin was removed, and the remainder of the chromatogram was washed with methanol. The extracted material was combined with all the chromatographic fractions not containing the compound of R_F 0.62, to make up fraction *G* (26 g).

Fraction *F* was recrystallized from acetone to yield colorless crystals which melted at 203° to 205°C; they showed a specific rotation ($[\alpha]_D^{33}$) of +63° in methanol solution, and ultraviolet absorption maxima at 216 mμ (ε 18,100) and 310 mμ (ε 40) in ethanol solution. The literature records the following physical constants for calotropin: mp 234° to 240°C, $[\alpha]_D^{25}$ +64° (methanol), $\lambda_{max}^{ethanol}$ 217 mμ (ε 17,800), 310 mμ (ε 35.5) (*9, 10*). There was no depression of melting point on admixture with authentic calotropin (mp, 198° to 205°C), and the infrared spectra of the respective samples (KBr pellets) could be superimposed. The respective samples showed identical R_F upon thin-layer chromatography on silica gel with 13 percent methanol in chloroform, and upon paper chromatography with chloroform-formamide. Similar fractionation of the extract of leaves of *A. curassavica* from Costa Rica also yielded calotropin.

Calotropin has previously been isolated from *Calotropis procera* R. Br. (Asclepiadaceae) (*11*) and from *Pergularia extensa* (Jacq.) N.E. Br. (Asclepiadaceae) (*10*). In a study of the cardenolides of *A. curassavica* L., Tschesche *et al.* (*12*) found seven aglycones, including calotropagenin, the aglycone of calotropin. Extraction under conditions known to minimize enzymatic hydrolysis yielded the glycoside uzarin (*13*). Calotropin is thus the second glycoside to be isolated from *A. curassavica* L., although no special precautions were taken in the present study to preclude enzymic hydrolysis during the extraction procedure. It is noteworthy that Reichstein *et al.* isolated calotropin from the seeds of *P. extensa* even when the material was extracted under "fermentation" conditions (*10*).

Calotropin was very recently assigned structure I (Fig. 2) by Hassall *et al.* (*14*); it thus bears a remarkable structural similarity to apocannoside (II) and cymarin (III), which have recently been shown to cause the cytotoxic activity of extracts of *Apocynum cannabinum* L. (Apocynaceae) (*15*).

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Thermoregulatory and Adaptive Behavior of Brown Adipose Tissue

Abstract. *Brown adipose tissue has been shown to be a strongly thermogenic effector organ in homeothermic animals exposed to cold and in hibernators during cold-induced arousal from deep hibernation. Because of the anatomical distribution of brown fat and the utilization of vascular countercurrent heat exchange, this cold-induced thermogenic response protects the animal by contributing heat to the vital organs of the thorax, the cervical and thoracic segments of the spinal cord, and the sympathetic chain. Evidence indicates that control of thermogenic activity of brown fat is mediated by the sympathetic nervous system.*

Brown adipose tissue was first described in 1551 by Conrad Gesner (*1*), who noted its appearance in the European marmot *Muris alpinus*. Since then scholars have further demonstrated that brown fat also occurs in many other species of rodents and, sporadically, in some five other orders of mammals, including man and other primates (*2*). From its initial discovery in the marmot, brown fat has consistently appeared in all true hibernators that have been studied, but its occurrence in many nonhibernators has increased the difficulty in assigning to it a functional role (*3*). Minimal criteria would rest on the finding of some common property by which the brown fat could be shown to be essential to survival of the hibernator and at least useful to the nonhibernator during some critical phase of its life cycle.