Occurrence of a cardiac heteroside in *Bersama yangambiensis*

SIR,—The presence of a cardiotonic bufadienolide has been demonstrated in leaves of *Bersama abyssinica* (Lock, 1962). This note gives evidence for the occurrence of a similar compound in the bark of another plant of the Bersama group—*Bersama yangambiensis* (Toussaint, 1959).

Crude extracts were prepared from various parts of *Bersama yangambiensis* by a modification of the method of Euw & Reichstein (1950). Tested on guinea-pigs, bark extracts were found to be about three times more toxic than leaf extract. Purification was achieved by successive extraction with diethyl ether, dichlormethane and dichlormethane–ethanol (3:2; v/v) followed by separation into eight fractions on an alumina column by eluents of increasing polarity. The last fraction, which retained the sour taste of the dichlormethane extract, was eluted by 94% ethanol–water (6:4; v/v) and was further characterized. Its dry residue developed characteristic colours when treated with concentrated sulphuric acid and was demonstrated to retain the bulk of the cardiotonic activity.

This chromatographically pure extract, readily soluble in ethanol, was shown to contain the steroidal skeleton of cardiotonic heterosides by well-established reactions (Lieberman, Baljet) described by Paech & Tracey (1955).

The spectral analyses were sufficiently conclusive to ascribe the bufadienolide structure to the compound. The infrared spectrum in chloroformic solution shows absorption bands at 1640^{-1} and 1605 cm^{-1} (C=C) and 1720 cm^{-1} (C=O) characteristic of the group CH=CH−C=O of the six-membered lactone ring of bufadienolide (Jones & Herling, 1954). However, the ultraviolet spectrum gives an absorption band at 279 mμ but not the bufadienolide band at 300 mμ. This may be explained by the presence of a ketonic function in position 11 or 12 of the cyclopentanophenanthrene molecule (Hegedus, Tamm & Reichstein, 1955).

The extract also possesses the pharmacological properties of cardiotonic heteroside as defined by Chen (1963). This sour-tasting compound stops the frog heart preparation of Straub in systolic contraction (Fig. 1A) and the

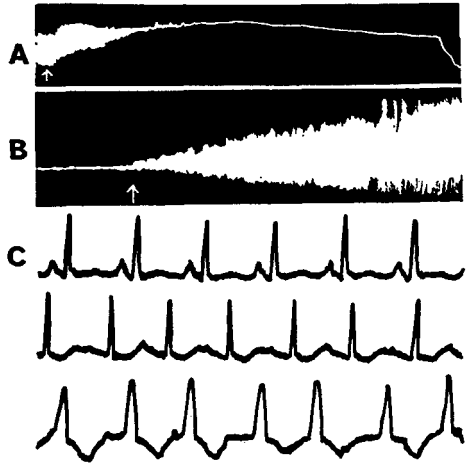

FIG. 1. A. Isolated frog heart perfused with a dilute solution of the Bersama extract. B. Rabbit auricle with addition of Bersama extract (about 200 μg is added to the 15 ml Locke solution in the organ bath). C. Electrocardiogram of a cat. Intravenous injection of 1·5 mg of a semi-purified extract at zero time (upper), 5 min after injection (middle) and 10 min after injection (lower record).

intravenous injection of the extract to the cat is followed 3–5 min later by auriculo-ventricular block, the degrees of the block increasing with time. Other rhythm disturbances including bigeminism were eventually observed (Fig. 1B). The action was over in 30 min but the maintenance of a myocardiac impregnation by the compound was evidenced by the faster and the more pronounced effect of a second injection.

Finally, the extract has a positive inotropic action on the isolated rabbit auricle preparation as described by Burn (1952) (Fig. 1C).

It is concluded that this evidence demonstrates the presence of a cardiotonic drug of bufadienolide type in *Bersama yangambiensis*.

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April 14, 1967

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A simple method for measuring the amount of azovan blue exuded into the skin in response to an inflammatory stimulus

SIR,—The most characteristic sign of inflammation is the enhancement of vascular permeability. To indicate the increased permeability, the sulphonic acid dyes like azovan (Evans) blue or trypan blue, which become bound to serum proteins, are suitable. The dye-protein complex is exuded into the surrounding tissues during the enhancement of the permeability. In most reports the evaluation of the local staining effect was confined to a subjective visual estimation such as colour intensity or diameter of the coloured areas. Several methods exist for extracting the dye from minced tissues, but they are complicated, especially for routine examinations (Sachs & Lummis, 1955; Clausen & Lifson, 1956; Judah & Willoughby, 1962; Hladovec, Horáková & Mansfeld, 1961). Of these methods only the last two have been elaborated for the extraction of the dye from the skin.

We now describe a simple extraction method in which a methanolic solution of suramin (Bayer 205) is used to extract the azovan blue at room temperature from a piece of skin without maceration or mincing.

The strong linkage of suramin to protein has been known for a long time (Mayer, 1922). Jancsó (1955) showed that the suramin molecules competed with the dye molecules for the possession of the binding sites in the tissue and in this way high concentrations of suramin displaced the bound dye from the tissue structures. This competition arises because the two compounds are similar in chemical constitution, both being symmetrical naphthalene sulphonic acids.

The suramin solution slowly elutes the dye from the skin so that within 14 days all the dye passes into the clear alcoholic phase and can then be measured spectrophotometrically. An Optica-Milan spectrophotometer is suitable.