counter. The radioactivity is registered with a ratemeter, preferably provided with a pulse-height analyzer, which keeps the background low and makes possible the analysis of the protein content of radioactive eluates, in our case macromolecular ^{57}Co-cyanocobalamin complexes. The readings of the ratemeter are registered graphically with a recorder. Before use, the column is washed with NaOH until the background reading becomes low. Using this system without a pulse-height analyzer we have found 0.45 μg of bovine albumin introduced in 1 ml volume to give noticeable peaks. With the use of better equipment and high-specific activity ^{64}Cu (not available to us due to the distance from the foreign manufacturer) it should be possible to detect still smaller amounts of protein. This system is more sensitive than the preceding one, since by this method the peak radioactivity values are recorded. For practical purposes the method is insensitive to ammonia, since only concentrated solutions elute radioactivity.

The Sephadex functions as a convenient filter and supporting medium, which effectively removes the originally dissolved copper as hydroxide and dissociates small-molecular copper complexes, and which can be prepared rapidly. The main disadvantage of the method is the short half-life of the copper isotope. A longer-lived isotope exists (^{67}Cu), but is not commercially available. On the macro scale our system also works with nickel, but the usual isotope ^{63}Ni emits solely soft beta radiation and is available only in low-specific activity form. Other metals which were tried gave disappointing results.

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Isothiocyanates XLIV *. The Isothiocyanate Glucoside (Glucocapparin) in *Crataeva Roxburghii* R.Br. *(Capparidaceae)*

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The tree *Crataeva Roxburghii* R.Br. (syn. *C. religiosa* Forst.) of the family *Capparidaceae* grows abundantly in the plains of India and affords a popular local remedy in the treatment of several physical and mental disorders. Of particular interest in connexion with current studies in this laboratory is the paper by Lahiri [1] according to which J. S. Chatterjee from "decomposed" bark of *C. Roxburghii* isolated a crystalline compound, $C_{15}H_{16}N_2S$, reported to be obtainable also from *Moringa pterygosperma* and horse-radish on similar treatment. The compound was demonstrated to be of considerable promise in the clinical treatment of cholera [1]. More recently, Chakravarti [2] established its structure as 1,3-dibenzylthiourea on basis of degradation experiments and comparison with a synthetic specimen **. Unfortunately, Chatterjee's isolation procedure never seems to have been published (*cf.* Ref.[2]). Hence, repetition under exactly the same conditions is not possible. In view of the present knowledge, however, it appeared likely that 1,3-dibenzylthiourea might originate from benzyl isothiocyanate, derivable from glucotropaeolin, a glucosidic progenitor widely distributed in the plant kingdom [4].

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** The same thiourea has been previously reported [3] as a constituent of the unsaponifiable matter from the seed fat ("Khakan fat") of *Salvadora oleoides* Den. (*Salvadoraceae*). On steam distillation, the fat gave about 1.5 % of benzyl isothiocyanate [3], and it appears likely that dibenzylthiourea in this case was an artifact produced from the mustard oil during saponification.

In this laboratory, bark material of *C. Roxburghii* R.Br.* was reinvestigated and quite different conclusions were attained. On paper chromatography of a methanolic bark extract for isothiocyanate glucosides only one spot was observed, indistinguishable in its R_F-value from that of a reference spot of glucocapparin [5], the predominant glucoside in most species of the family *Capparidaceae* as evident from reported data [6] and a considerable unpublished material in this laboratory. No trace of glucotropæolin was detectable in the bark extract. The alcoholic extracts gave no reaction for thioureas when tested with Grote's reagent.

On enzymic hydrolysis of the methanolic glucoside extract only one isothiocyanate was produced, identified as methyl mustard oil, the expected product from glucocapparin, on conversion into thiourea and paper chromatography of the latter. Finally, an infra-red spectrum served to confirm the identity of the isolate with 1-methylthiourea.

Investigation in this laboratory of an extract of dried leaves of *C. Roxburghii* again revealed exclusively the presence of glucocapparin yielding methyl isothiocyanate on enzymic hydrolysis.

Hence, we have not been able to confirm the presence of 1,3-dibenzylthiourea or any likely precursor of this compound in *C. Roxburghii* and we can offer no explanation for the results reported from Indian laboratories [1,2].

Experimental. Finely pulverized bark of *C. Roxburghii* (200 g) was refluxed for 2 h with 2 l of 70 % methanol, and the extraction was repeated. On paper chromatography in butanol:ethanol:water (4:1:4) and spraying with silver nitrate, this extract gave only one glucoside spot, indistinguishable from that given by an authentic specimen of glucocapparin.

The filtrate was concentrated *in vacuo* (to 450 ml) and filtered through Celite. The solution was buffered at pH 6 with citrate; a trace of ascorbic acid [7] and a myrosinase solution (25 ml) were added, and the enzymic hydrolysis was allowed to proceed for 42 h. Half of the hydrolysis mixture was subjected to steam distillation, the distillate was treated for 3 h with 4 N ammonia, and the solution was evaporated to dryness. The other half was extracted twice with ether and twice with chloroform. The combined extracts were dried and the main part of the solvents were removed over a column. The residue was treated with a solution of ammonia in chloroform for 3 h, and the solvent was removed. Both preparations on paper chromatography in (*a*) water-saturated chloroform, and (*b*) butanol:ethanol:water (spray: Grote's reagent) showed the same pattern, *viz.* one thiourea indistinguishable from a simultaneously run reference sample of methylthiourea.

The combined thiourea preparations were dissolved in a few ml of ethanol and passed through a layer (3 cm) of neutral alumina to remove some yellow contaminations by means of ethanol. The semi-crystalline residue was triturated with a few drops of chloroform and ether and the purified material was employed for infra-red spectroscopy in a KBr-disc. The spectrum was indistinguishable from that of authentic methylthiourea.

A methanolic extract of dried leaves of *C. Roxburghii* was produced in a Waring blendor. Disturbing impurities were removed on precipitation with lead acetate and the purified glucoside solution was chromatographed in the usual way. Only one spot, identical in R_F-value with glucocapparin, was observed. An enzymically hydrolyzed glucoside solution was steam-distilled, converted into thiourea, and chromatographed as described above. Again, only one spot was produced, indistinguishable from that of methylthiourea.

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