SHORT COMMUNICATION

COMPLEXES OF COPPER WITH SOME PYRROLIZIDINE ALKALOIDS AND WITH SOME OF THEIR ESTERIFYING ACIDS*

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Bull *et al.* (1956) and Bull and Dick (1959) have shown that the repeated intake of pyrrolizidine alkaloids of the plant *Heliotropium europaeum* can lead to severe liver damage, and to the abnormal accumulation of copper in the livers of sheep and rats.

The possibility of complex formation between copper and pyrrolizidine alkaloids was investigated in the present study.

Methods and Results

(i) *Complexes at Alkaline pH.*—2N NaOH was added to a series of solutions of pyrrolizidine alkaloids in aqueous $CuSO_4$ until pH 12 was reached. $Cu(OH)_2$ was allowed to settle, and was then centrifuged off. The colour of the supernatants was compared with a solution of $CuSO_4$ brought to pH 12 with 2N NaOH. A characteristic dark blue colour was detectable over a wide range of proportions of alkaloids to $CuSO_4$ in the case of those alkaloids which were esters of an organic acid which had two or more hydroxyl groups on adjacent carbon atoms (Table 1).

Solutions of the free esterifying acids, lasiocarpic, trachelanthic, and viridifloric acids, all of which contain an α-glycol group, gave the characteristic colour with Cu^{++} at alkaline pH, but a solution of heliotric acid, which does not have an α-glycol group, did not give the colour.

(ii) *Molecular Proportions of the Complexes at Alkaline pH.*—Solutions of different concentrations of the alkaloids lasiocarpine, lasiocarpine *N*-oxide, and monocrotaline or of lasiocarpic and trachelanthic acids were prepared, and the same amount of $CuSO_4$ added to each. NaOH was added to pH 12 for the alkaloids and to pH 10 for the acids, and the solutions were adjusted to the same volume with water and filtered. Extinction coefficients at 640 mμ, the approximate absorption maximum of all the filtrates, were read against a blank which originally contained the same amount of $CuSO_4$ and which was treated in the same way.

It was found by plotting the extinction coefficients that monocrotaline, lasiocarpine, lasiocarpine *N*-oxide, and trachelanthic acid combine with copper in the molar proportion of 2 : 1, and that lasiocarpic acid and copper appear to combine in the ratio of 1 : 1. The amount of copper involved in complex formation with lasiocarpic acid may, however, be exaggerated by salt formation with the carboxylic acid group which is not free in the alkaloid.

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(iii) *Effect of pH on Complex Formation.*—When a solution of equimolar proportions of lasiocarpic acid and $CuCl_2$ was titrated with 0·1N NaOH, the extinction coefficient at 640 mμ, read against a water blank and corrected for increases in

TABLE 1
COLOUR TEST WITH ALKALOIDS AND CUPRIC IONS AT ALKALINE pH

Alkaloid	Result	No. of Hydroxyl Groups in Esterifying Acid	Alkaloid	Result	No. of Hydroxyl Groups in Esterifying Acid
Echimidine	Positive	3*	Heliotrine	Negative	1
Echinatine	,,	2*	Heliotrine *N*-oxide	,,	1
Heliosupine	,,	3*	Platyphylline	,,	1
Lasiocarpine	,,	2*	Senecionine	,,	1
Lasiocarpine *N*-oxide	,,	2*	Seneciphylline	,,	1
Supinine	,,	2*	Spectabiline	,,	1
Supinine *N*-oxide	,,	2*			
Monocrotaline	,,	2*			

* On adjacent carbon atoms.

volume, was found to increase as the pH rose through the range 4–9 approximately (Fig. 1), indicating that complex formation began at about pH 4.

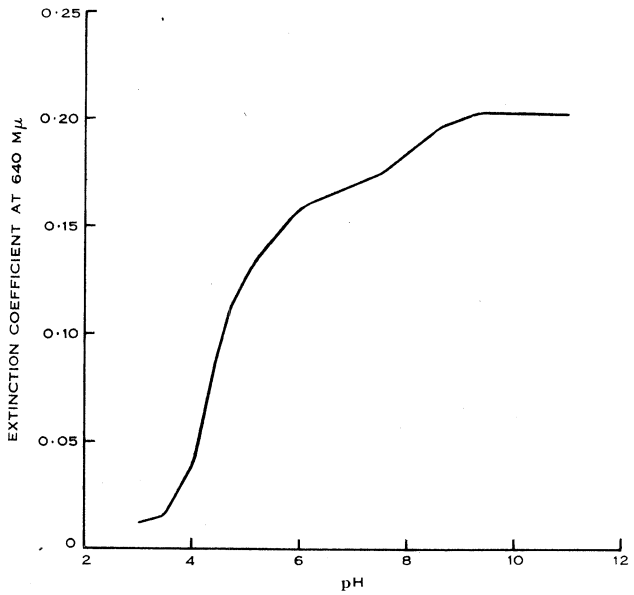

Fig. 1.—Titration of an equimolar solution of lasiocarpic acid and $CuCl_2$ with 0·1N NaOH.

Further evidence on the formation of organic acid–copper complexes was obtained by following the change in pH of solutions, which resulted from the

addition of NaOH. Figure 2 shows how the mixtures of lasiocarpic acid and $CuCl_2$, heliotric acid and $CuCl_2$, and trachelanthic acid and $CuCl_2$, in the molar ratios of 1 : 1, 2 : 1, and 2 : 1 respectively, altered the pH titration curves from those resulting from titration of the acids and $CuCl_2$ separately. Clearly heliotric acid, also, combined with $CuCl_2$.

(iv) *Copper Complexes at pH 6.*—When alkaline solutions of alkaloids and copper were treated with acid to lower the pH, precipitation occurred between pH 9 and pH 6. Consequently it was not possible to study complex formation between copper and the complete alkaloids at pH 6.

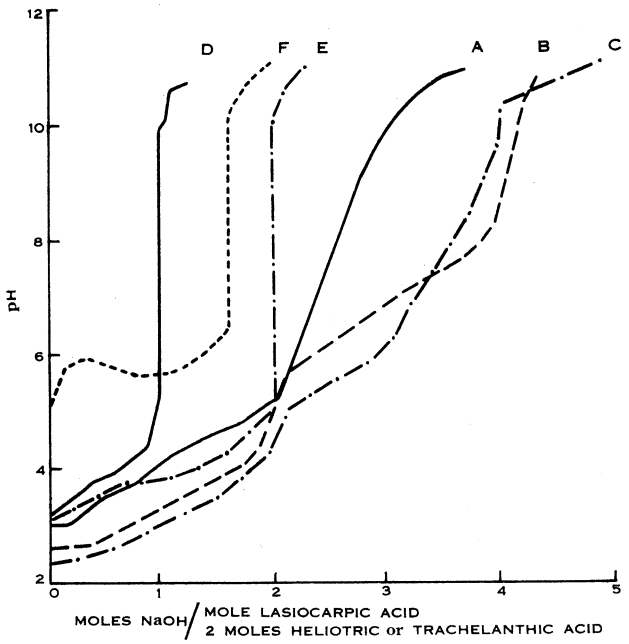

Fig. 2.—Titration with 0·1N NaOH of: *A*, lasiocarpic acid (100 mg) and $CuCl_2$, molar ratio 1 : 1; *B*, heliotric acid (100 mg) and $CuCl_2$, molar ratio 2 : 1; *C*, trachelanthic acid (100 mg) and $CuCl_2$, molar ratio 2 : 1; *D*, lasiocarpic acid (100 mg); *E*, heliotric or trachelanthic acid (100 mg); *F*, $CuCl_2$—same amount as for *B*.

Precipitation did not occur from solutions of the organic acids and copper at pH 6 and so the formation of acid–copper complexes at this pH was investigated. Solutions of lasiocarpic, trachelanthic, or heliotric acids and $CuSO_4$ at pH 6 were blue-green in colour and absorbed light maximally between 710–760 mμ. Measurement of the extinction coefficients at the absorption maxima in solutions of different molecular proportions showed that at pH 6, lasiocarpic acid combines with copper in the molar ratio of 1 : 1, and that trachelanthic and heliotric acids combine with copper in the ratio 2 : 1. Precipitation occurred from solutions of heliotric acid and copper above pH 7, and with trachelanthic acid and copper above pH 9, on standing. No precipitation occurred with lasiocarpic acid and copper below pH 10.

Discussion

The alkaloids of *H. europaeum* which contain an esterified acid with an α-glycol group, and the free α-hydroxy acids of these alkaloids, have been found to form complexes with copper. Angelic acid, the only acid associated with the pyrrolizidine alkaloids of *H. europaeum* which does not have an α-hydroxy group, was not tested. In view of the abnormal accumulation of copper in the liver of sheep which have grazed *H. europaeum*, and the similar accumulation associated with the administration of pyrrolizidine alkaloids to rats, these complexes may be of biological interest. It is not possible, on present published information, to determine whether liver damage by the alkaloids is a prerequisite for abnormal accumulation of copper. It may be that copper is accumulated as a result of complex formation either with the alkaloids or with the free acids after *in vivo* hydrolysis, and it may be purely chance that increased levels of copper were found only after liver damage was evident (Bull and Dick 1959).

Acknowledgment

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References

Bull, L. B., and Dick, A. T. (1959).—*J. Path. Bact.* **78**: 483.

Bull, L. B., Dick, A. T., Keast, J. C., and Edgar, G. (1956).—*Aust. J. Agric. Res.* **7**: 281.