

An extraction procedure was devised which excluded the interfering substances but gave a low recovery of histamine. This involved a combination of ion exchange (Huff, Davis & Brown, 1966) and solvent extraction (Shore, Burkhalter & Cohn, 1959): plant material was extracted with N trichloroacetic acid, the extract adjusted to pH 7.5 and passed through Amberlite CG 50 anionic exchange resin, the eluate was subjected to solvent extraction before the fluorogenic reaction with oPT.

The histamine content of fresh tissue of *Gossypium* species was estimated by this procedure: *G. hirsutum*, old leaf, 87 µg histamine/g fresh leaf; *G. hirsutum*, young mature leaf, 101 µg/g; *G. arboreum*, young mature leaf, 113 µg/g. Fresh mature bracts of *G. hirsutum* were estimated to contain 26 µg histamine/g of *G. arboreum*, 6 µg histamine/g.

By comparison, dried leaf of *G. hirsutum* was estimated to contain 1760 µg histamine/g which corresponded to 330 µg histamine/g fresh leaf.

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The estimation of rauwolfia alkaloids by quantitative thin-layer chromatography

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Previous work on the estimation of rauwolfia alkaloids (Harris, Stewart & Court, 1968; Los & Court, 1969) involved thin-layer chromatographic separation followed by ultraviolet spectrophotometric estimation of the eluted alkaloids. Substances extracted from the adsorbent are known to interfere with the spectrophotometric measurements (Harris, 1966) and coloured complexes of the alkaloids can be formed and measured at wavelengths such that interference is minimal (Court, 1968).

Table 1. *Alkaloid content of Rauwolfia root bark.*

| Alkaloid | <i>R. caffra</i> | | <i>R. vomitoria</i> | |
|--------------|-----------------------------|--------------------------|------------------------------|--------------------------|
| | Mean percentage | Coefficient of variation | Mean percentage | Coefficient of variation |
| Ajmalicine | 0.016 | 2.20 | — | — |
| Ajmaline | 0.239 | 1.17 | 0.090 | 3.33 |
| Rescinnamine | 0.013 | 1.43 | 0.105 | 1.33 |
| Reserpiline | — | — | 1.090 | 1.01 |
| Reserpine | 0.016 | 1.97 | 0.218 | 0.46 |
| Serpentine | 0.180 | 1.24 | — | — |
| | (based on 5 determinations) | | (based on 10 determinations) | |

In this work 10 rauwolfia alkaloids were separated using various chromatographic systems employing silicagel G layers 250 µm thick. The individual alkaloids were recovered by elution in alkaline chloroform and complexed with iodine in citrate-phosphate buffer (pH 4.1). The absorption measurements of the alkaloid-complex solutions were recorded at appropriate wavelengths in the range 365-396 nm. Results were calculated from compensated standard curves.

Recovery of the alkaloids by elution from the plates was investigated and the method was applied to samples of *Rauwolfia caffra* and *R. vomitoria* root barks (Table 1).

The method is more rapid and accurate than the earlier method and yields lower results due to reduced interference.

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