The demonstration of a tyramine-like substance in a heart extract (Recosen)

A protein-free total heart extract, available in both tablet and ampoule form, has been reported to be useful in a number of cardiovascular disorders (Karani & Elliott, 1961; Rey & Pattani, 1954). The extract (Recosen) has been reported to contain histamine, adenosine derivatives and various cations (Conway, 1959), while Meyer & Goldberg (1966), using pharmacological techniques, identified a tyramine-like substance. Using chromatography, we have found tyramine in the extract. We have also found that the whole of the cardiotonic activity is accountable for by this amine.

The contents of 30 one ml ampoules were pooled, concentrated and the concentrate dialysed. The dialysate was chromatographed through a column packed with Biogel P2 (Biorad) polyacrylamide gel equilibrated with 0·1 N acetic acid. The fractions eluted from the column were dried below 30° and bioassayed for positive inotropic activity using the isolated papillary muscle of the cat (Thorp & Cobbin, 1967).

The constituents of the extracts were separated by ascending paper chromatography on Whatman No. 1 paper using three solvent systems, and the results visualized by a diazotisation spot test for amines (Dawson, Elliot & others, 1969a), ninhydrin 0·5% in n-butanol, and an alkaline Folin-Ciocalteau reagent for phenols (Dawson, Elliott & others, 1969b). Catecholamines were detected with a potassium ferricyanide spray in phosphate buffer (James, 1948). Some paper chromatograms were cut into segments which were eluted for bioassay. Tyramine, and β-phenethylamine, another indirectly acting sympathomimetic amine occurring in tissue extracts (Jackson & Temple, 1970), were used as controls. Table 1 lists the results.

In each solvent system, two components of the extract were detected by each of the stain techniques used, one having a relatively high and the other a relatively low Rf value, indicating the presence of two phenolic amines. The Rf value for tyramine in all solvent systems was similar to that of the more mobile component in the extract. Moreover, elution and bioassay of the chromatogram in segments indicated that the positive inotropic activity of the extract, which apparently remained a single component in each solvent system, had a similar Rf to tyramine in each solvent system. The intensity of the colour produced with the diazotization or Folin-Ciocalteau reagents by the more mobile spot when 250 μg of extract was chromatographed,

Table 1. *Rf values for components of heart extract compared to tyramine and phenethylamine, using various reagents*

Reagent	Solvent system a	b	c
	Rf values		
Ninhydrin	0·17 MP	0·48 P	0·34 P
		0·63 B	0·67 B
Diazo	0·16 P	0·47 C	0·35 CP
	0·52 C	0·71 C	0·70 P
Folin	0·17	0·44	0·32
	0·50	0·67	0·68
Activity on bioassay of eluted chromatogram	0·27–0·41	0·5–0·7	0·5–0·6
Tyramine (average)*	0·43	0·66	0·64
β-phenylethylamine	0·56	0·85	0·73

a = n-Butanol–water (86:14).
b = n-Butanol–acetic acid–water (12:3:5).
c = n-Butanol–methanol–water (5:5:1).
M = Mauve, P = purple, C = crimson.
* Detected by each reagent and by elution and bioassay.

was comparable to the colour intensity of 0·5 to 1·0 μg tyramine standards under the same conditions. Bioassay of 1 μg of tyramine gave an average value for cardiotonic activity for ten assays on the isolated cat papillary muscle of 203% $\pm$ 40, while the assay of 250 μg of extract before chromatography gave a result of 245% $\pm$ 47 for five assays, the results being expressed as mean $\pm$ standard error. Noradrenaline, adrenaline, dopamine, isoprenaline, histamine, aldosterone, 5-hydroxytryptamine and phenylalanine were also chromatographed in the same systems but none had the same or similar Rf values to the extract in all the systems. These findings suggest that all the cardiotonic activity of the extract is accountable for on the basis of its tyramine content. The extracts were inactive when assayed on muscle preparations from reserpinized cats, confirming the indirectly-acting nature of the extracts. No noradrenaline, adrenaline or dopamine were detected by the ferricyanide spot tests, but a ninhydrin-reacting constituent corresponding in Rf to histamine was detected in the extracts occasionally.

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The isolation and identification of (–)-apparicine from *Tabernamontana cumminsii*

Conopharyngine, the major alkaloidal component (Thomas & Starmer, 1963; see also Renner, Prins & Stoll, 1959), jollyanine (conopharyngine hydroxyindolenine) (Crooks & Robinson, 1970) and 2-ethyl-3-[2-(3-ethylpiperidino)-ethyl] indole (Crooks, Robinson & Smith, 1968) have already been isolated from the ether-soluble bases obtained from the leaves of *T. cumminsii*. We have now identified a fourth alkaloid, m.p. 188–191°, $[\alpha]_D^{22} = -170 \pm 10°$ (in $CHCl_3$), whose isolation from this source we have already reported (Crooks & Robinson, 1970).

The high resolution mass spectrum of the alkaloid showed a molecular ion at m/e = 264·162387 which indicated a molecular formula $C_{18}H_{20}N_2$ (calculated 264·162641). Apart from the molecular ion, which was also the base peak, the mass spectrum had other significant peaks at m/e = 249, 235, 222, 208, 194, 180, 167, 154, 130 and 128. The ultraviolet spectrum in ethanol had λ_{max} 303–305 nm (log ϵ = 4·65), λ_{infl} 309–312 nm (log ϵ = 4·60), λ_{min} 268 nm (log ϵ = 3·78) which did not change upon acidification.