SIMPLE HYPOTENSIVE AND HYPERTENSIVE PRINCIPLES FROM SOME WEST INDIAN MEDICINAL PLANTS

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Examination of those plant extracts which were previously found (Feng and others, 1962) to have transient depressor activity has led to the isolation of γ-aminobutyric acid, from the leaves of *Artocarpus incisa* L. and of *Piper amalago* L. and to the demonstration by paper chromatography of its presence in several extracts. Extracts showing pressor activity were also examined and tyramine has been isolated from *Phoradendron wattii* Kr. and Urb. and dopamine has been shown by paper chromatography to be present in *Piper amalago* L. and *Stachytarpheta jamaicensis* Vahl.

SEVERAL extracts obtained from West Indian medicinal plants were found to affect the blood pressure when injected into a dog anaesthetised with pentobarbitone sodium (Feng and others, 1962). These extracts have been examined in more detail to determine what substances were responsible for the effects: we now report the identification of γ-aminobutyric acid, tyramine and dopamine in various extracts.

RESULTS

γ-Aminobutyric acid. Several of the extracts showed quite marked transient depressor effect on the blood pressure of the pentobarbitone anaesthetised dog. In two such experiments, the extracts from the leaves of *Artocarpus incisa* L. ("breadfruit") and from those of *Piper amalago* L. ("pepper elder"), the factor responsible was isolated by using ion-exchange resins and thick paper chromatography. It was identified as γ-aminobutyric acid by paper chromatography, infra-red spectrum, mixed melting point, and by preparation of derivatives. This substance has been shown to have a transient depressor effect when injected into laboratory mammals (Iino, 1955, Takayasu, 1956) and man (Elliott and Hobbiger, 1959).

Other extracts which showed qualitatively similar transient depressor effects were examined by two dimensional paper chromatography for the presence of γ-aminobutyric acid. By this procedure, extracts from the following plants were found to contain it.

Agave angustifolia Haworth Var. Marginata.
Alchornea latifolia Sw. ("loblob", "sweet wood").
Andira inermis H.B.K. ("Cabbage bark").
Annona muricata L. ("Soursop").
Cassia occidentalis L. ("Wild dandelion").
Cayaponia racemosa Cogn. ("Wild cerassee").
Cocos nucifera L. ("Coconut")—mature liquid endosperm.
Croton linearis Jacq. ("Wild rosemarie", "Spanish rosemarie").
Cuscuta americana L. ("Love bush").

Forsteronia floribunda (Sw.) A.DC.
Momordica charantia L. ("Cerassee").
Oryctanthus occidentalis Eichl ("Mistletoe").
Phoradendron rubrum Griseb. var. gracile ("Mistletoe").
Piper auritum H.B.K.
Samanea saman Merrill ("Guango").
Sechium edule Sw. ("Cho'cho").
Stachytarpheta jamaicensis Vahl. ("Vervine").
Solanum torvum Sw. ("Susumba").
Tribulus cistoides L. ("Police macca").
Zebrina pendula Schnizl. ("Red water grass").

Tyramine. The strong pressor effect on the blood pressure of the pentobarbitone anaesthetised dog observed in extracts of *Phoradendron wattii* Kr. and Urb. ("mistletoe") was found to be associated with a substance which behaved similarly to tyramine on paper chromatography. The substance was isolated by extraction, alumina chromatography, and crystallisation. It was identified as tyramine by infra-red spectrum and mixed melting point. Tyramine has been reported to be present in other *Phoradendron* species (Crawford and Watanabe, 1914 and 1916).

Dopamine. Strong pressor effects were also observed in extracts of *Piper amalago* L. and *Stachytarpheta jamaicensis* Vahl. These extracts were shown to contain a substance which was identical with dopamine on paper chromatography and the pressor activity is almost certainly caused by the presence of dopamine.

Experimental

Isolation of γ-Aminobutyric Acid from Artocarpus incisa *L.*

Freshly picked mature leaves (2 kg.) were macerated in a Waring Blendor with water (10 litres) then boiled for 1 hr. and filtered through fine cloth. The residue was again extracted with water (8 litres) and the combined filtrates were concentrated under reduced pressure to a volume of 1·2 litres. Ethanol (3·6 litres) was added to precipitate protein and the solution left standing for 2 hr. at 4° then filtered. The filtrate after concentration under reduced pressure to 1 litre was passed through a column of Amberlite IR 120(H^+) (780 g.). The resin was washed with water until the eluate no longer gave a colour with ninhydrin and then with 0·1N ammonium hydroxide. The ammoniacal eluate but not the aqueous showed hypotensive activity.

Evaporation of the active solution to dryness under reduced pressure gave a brown solid (7·9 g.) which on paper chromatography in butanol : acetic acid : water (4 : 1 : 5) was found to contain eight ninhydrin positive spots. The activity was associated with the spot running with R_F 0·36 and the material (305 mg. from 2·7 g. crude solid) was isolated by elution of the appropriate zone of chromatograms run on Whatman 3 MM and Whatman Seed Test (Brownell, 1957) papers in the same system. Three crystallisations from ethanol using charcoal gave colourless needles (52 mg.) m.p. 192–197°. The infra-red spectrum was identical with that

of authentic γ-aminobutyric acid, with which no depression was observed on mixed m.p. determination. Chromatographic behaviour was also identical with an authentic sample and no differences were found on two dimensional chromatography on Whatman No. 1 paper in phenol : water (4 : 1) and collidine : lutidine : water (1 : 1 : 1); on circular chromatography (Saifer, 1956) in butanol : acetic acid : water (4 : 1 : 5) and in phenol : water (4 : 1); and on dusting the origin of chromatograms developed in phenol : water (4 : 1) with basic copper carbonate (Crumper, 1949) which forms complexes with α-amino-acids but does not affect γ-aminobutyric acid. A *p*-toluene sulphonyl derivative was prepared, m.p. 137–139° undepressed on admixture with authentic *N*-(*p*-sulphonyltoluene) γ-aminobutyric acid.

Isolation of γ-Aminobutyric Acid from Piper amalago *L.*

γ-Aminobutyric acid was isolated from the leaves of this plant by the method described in the previous section. Additional criteria for its identity were: the comparative behaviour on paper chromatography on Whatman No. 1 paper in the systems: methyl ethyl ketone : isopropanol : water (2 : 2 : 1); t-butanol : 2N ammonium hydroxide (2 : 3); phenol (25 g.) : *m*-cresol (25 g.) : borate buffer pH 9·3 (7 ml.); and in the two dimensional systems of Levy and Chung (1953) which showed the isolated material to be identical with an authentic sample. Further evidence for this identity was given by the behaviour on electrophoresis by the horizontal technique of Grassman and Hannig (1950). On Whatman No. 1 paper, at pH 3·5 and pH 6·5 (pyridine acetate buffer) with a current of 25 mA at 250 V no difference in mobility was observed. A 2,4-dinitrophenyl derivative m.p. 146–147° was prepared, identical with 2,4-dinitrophenyl γ-aminobutyric acid.

Paper Chromatographic Screen for γ-Aminobutyric Acid

3 ml. of every standard solution which showed a transient hypotensive response was shaken with Dowex-50-X8, 100 mesh, H^+ form (3 g.) for 30 min. The resin was filtered off, washed with water (3 × 5 ml.) and the amino-acids eluted with 4 × 3 ml. of 3N ammonia solution. The solution was brought to dryness under reduced pressure, made up to 3 ml. with water and applied to one corner of Whatman No. 1 paper (20 $cm.^2$) as (5–10) × 5 μl. spots as the hydrochloride. Papers were run in pairs, to one of which 25 μg. each of proline and γ-aminobutyric acid were added, and accompanied by a marker paper with proline (which provides a useful guide to the γ-aminobutyric acid position) and γ-aminobutyric acid. These were run with occasional full marker sheets with one of two standard mixtures of amino-acids. Mixture A: leucine, proline, phenylalanine, valine, tryptophan, threonine, glycine, aspartic acid and lysine. Mixture B: isoleucine, methionine, tyrosine, alanine, glutamic acid, serine, cystine, histidine and arginine. Ascending runs were carried out in two dimensions [first system: butanol : acetic acid : water (4 : 1 : 5); second system: phenol (25 g.) : *m*-cresol (25 g.) : borate buffer pH 9·3 (7 ml.)]. After the papers had been dried thoroughly, colours were

developed by dipping the papers into 0·25 per cent ninhydrin in acetone then heating to 80° for 5 min.

Isolation of Tyramine from Phoradendron wattii *Kr. and Urb.*

Ten Whatman 3 MM papers ($18\frac{1}{4} \times 22\frac{1}{2}$ in.) were loaded with standard deproteinised solution (circa 1 ml. each) in a narrow (0·6 mm.) band and run in butanol : acetic acid : water (4 : 1 : 5). Horizontal strips were arbitrarily cut, eluted with warm water and tested in a pentobarbitone anaesthetised dog. The strip corresponding to R_F 0·60–0·62 showed pressor activity and the material from this zone gave a positive coupling test with diazotised sulphanilic acid for phenols and a positive fluorescein chloride test for primary amines.

280 g. of dried, milled plant was extracted with methanol in a Soxhlet apparatus for 24 hr. The solvent was evaporated under reduced pressure and the dry residue digested with 10 per cent hydrochloric acid (1 litre) for 24 hr. The solution was continuously extracted with chloroform for 15 hr. to remove neutral material, then made just basic with ammonia and extracted further with fresh chloroform. Evaporation of the solvent gave crude base (0·3 g.) which was purified by adsorption on an alumina column (30 g.) from which it was eluted with acetone. The resulting material ran with a single spot (R_F 0·61) on Whatman No. 1 paper in the *n*-butanol : acetic acid : water (4 : 1 : 5) system, and formed a crystalline hydrochloride with an infra-red spectrum identical with that of authentic tyramine hydrochloride and giving m.p. and mixed m.p. 269°.

Paper Chromatographic Identification of Dopamine in Piper amalago *L. and* Stachytarpheta jamaicensis *Vahl.*

The pressor activity in these plants was associated with bands of R_F circa 0·31 in the butanol : acetic acid : water (4 : 1 : 5) system. The reaction of the materials in these bands with potassium ferricyanide in buffer pH 7·8 (James, 1948) gave a colour characteristic of the sympathomimetic catecholamine type.

1·38 litres of either plant extract prepared by boiling fresh crushed plant material (650 g.) with water (2·38 litre), was introduced to a column of Dowex-50-X4 (Bertler, Carlsson and Rosengren, 1958) (H^+ form, 100–200 mesh) (30 × 4 cm.) and allowed to percolate (drip rate 500 ml./hr.). The column was then washed with N hydrochloric acid and the initial eluate (circa 2·2 litres) was discarded. The next fraction (total volume 600 ml.) which gave a positive reaction with the potassium ferricyanide reagent was collected, concentrated under reduced pressure at 45° to 100 ml. and examined by paper chromatography. Spots (50 μl.) were applied to Whatman No. 1 paper and chromatography in the following systems:

	R_F
Phenol : water (atmosphere of HCl)	0·49
Butanol : acetic acid : water (4 : 1 : 5)	0·31
Methyl ethyl ketone : water	0·71
Methanol : pentanol : benzene : water (2 : 1 : 1)	0·57
Methanol : water (1 : 1)	0·16

The R_F values of the spot the spot obtained with potassium ferricyanide reagent, are identical to those given by authentic dopamine, which was run as marker. Dopa, Epinine, Corbasil, adrenaline and noradrenaline were also run as markers.

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