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CARICACEAE

THE BASIC CONSTITUENTS OF THE LEAVES OF *CARICA PAPAYA**

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Abstract-The alkaloid carpaine has been isolated from the leaves of the Nigerian variety of *Carica papaya* L and some new derivatives of the alkaloid have been prepared. It was found that the yield of this alkaloid from the Nigerian plants is markedly lower than the values reported for American and Asian varieties. Also the plants were found to have a relatively high content of choline, and the procedure used for isolating choline is suggested as a simple method for separating choline from nitrogenous base mixtures (particularly mixtures containing other less strongly basic quaternary amines) in biological materials.

INTRODUCTION

THE MAJOR alkaloid present in the leaves of *Carica papaya* L. is carpaine[1-3] and the only other nitrogenous base reported hitherto from the plant is 'pseudocarpaine', a stereoisomer of carpaine found in very small quantity by Govindachari *et al.*[3] In connection with a radiochemical study[4] of the biogenesis of carpaine, it became necessary to process local *Carica papaya* ('paw-paw') leaves in order to isolate the alkaloid for use in isotope dilution. The initial yields of the alkaloid were so much lower than the values reported in the literature for Asian and American varieties that it seemed worthwhile to investigate whether the low yields obtained were due to some seasonal fluctuation in carpaine content or was characteristic of the local 'paw-paw' plants. C. *papaya* leaves were, therefore, collected monthly for a whole year from the local bush, and their carpaine contents were estimated.

With large quantities of C. *papaya* leaves thus available it appeared desirable to re-examine the leaves altogether for the possible presence of quaternary alkaloids which could be responsible for some of the curative properties attributed to the plant, particularly in traditional gynaecology.[5,6]

RESULTS AND DISCUSSION

Preliminary attempts to isolate carpaine from C. *papaya* leaves by the method of Govindachari et al. yielded an impure product which could not be induced to crystallize.

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† The work reported here was done in the Department of Chemistry, University of Ibadan, Nigeria,

[1] G. BARGER, R. ROBINSON and T. S. WORK, *J. Chem. Soc.* 711 (1937).
[2] H. RAPOPORT and H. D. BALDRIDGE, *J. Am. Chem. Soc. 73, 343 (1951).*
[3] T. R. GOVINDACHARS, B. R. PAI and N. S. NARASIMHAN, *J. Chem. Soc.* 1847 (1954).
[4] C. W. L. BEVAN and A. U. OGAN, *Phytochem. 3, 591 (1964).*
[5] J. M. DALZIEL, *The Useful Plants of West Tropical Africa,* p. 52, Crown Agents, London (1937).
[6] J. M. WATT and M. G. BREYER-BRANDWIJK, *The Medicinal and Poisonous Plants of Southern and Eastern Africa,* 2nd Edn, p. 167, Livingstone, Edinburgh & London (1962).

It was purified further via the reineckate complex.[7] The final yield of carpaine was 0·01 15 % of the dry weight of the leaves, as against yields of up to 0·2% reported for Malayan plants. The yield of carpaine remained uniformly low through the twelve successive monthly estimations, and it was not improved even by the use of younger leaves as suggested by Barger *et al.*[1]

Since very little information is available in the literature about the derivatives of carpaine, attempts were made to prepare some such derivatives using the conventional alkaloid reagents, but with only limited success.

To isolate the quaternary alkaloids, the leaves were first freed from carpaine by exhaustive extraction with chloroform in a Soxhlet. The residual plant material was next extracted with methanol. Pharmacological tests (carried out by staff of the Department of Pharmacology, University of Ibadan) showed that the residue obtained by distilling off the methanol had an oxytocic effect *in vitro* on guinea-pig uterus, but not on similar rat tissue. Paper chromatographic examination revealed that the 'alkaloidal' component of this methanol extract was comprised mostly of choline. The choline was isolated and estimated by making use of its preferential absorption on the cation-exchange resin Amberlite IRC-50. The yield of choline was 0·02% of the dry weight of the leaves. The choline was characterized further via its chloroaurate.

The results indicate that the Nigerian variety of C. *papaya* is relatively low in carpaine content and that choline is in fact a more abundant basic constituent of this source than is carpaine. It is well-known, of course, that choline occurs in several plants,[8–10] but only in a few instances[11] has it been detected in more than trace amounts. The *in vitro* oxytocic effect of C. *papaya* methanol extract on guinea-pig uterine tissue is probably related to the well-known stimulant action of acetylcholine on smooth muscle.[12] The significance of this as regards some of the alleged medicinal properties of C. *papaya* is uncertain, in view of the finding that rat uterine tissue (which usually approximates closely to human uterine tissue in its response to oxytocic drugs) was unaffected by this extract.

The isolation procedure employed here for choline (i.e. by utilizing its preferential adsorption on Amberlite IRC-50) is suggested as an uncomplicated method of separating this compound from nitrogenous base mixtures (particularly mixtures containing other quaternary bases) in biological materials.

EXPERIMENTAL

(1) General

Materials and methods. **The *Carica papaya* leaves were collected as required from the local bush in Ibadan. Only two estimations of carpaine are reported here in detail because the values obtained from the other ten estimations were comparable. M.ps were determined with a 'Rotax' Kofler-block type of apparatus (Albert Balzer, Basel, Switzerland) and are corrected. The ion-exchange chromatographic resins (B.D.H.) were used as described in the literature.[13,14] Paper chromatography was by the descending method on Whatman's No. 1 paper using the solvent system of Partridge and Westall.[15] The spots were identified by**

[7] E. STRACK and K. FOSTERLING, *Ber. dtsch. chem. Ges.* 76B, *14 (1943).*
[8] *G.* KLEM and A. ZELLER, *Chem. Zbl. II,* 1104 (1930).
[9] G. KLEIN, M. KRISCH, G. POLLAUF and G. Soos, *Chem. Zbl.* I, 847 (1932).
[10] R. PARIS and H. MOYSE-MIGNON, *Ann. pharm. franc. 14, 464 (1956).*
[11] H. BORUTTAU and H. CAPPENBERG, *Arch. pharm. Berl. 259, 33 (1921).*
[12] D. H. L. EVANS and H. 0. SCHILD, *Nature, Lond.* 180, 341 (1957).
[13] H. BAGGESGAARD-RASMUSSEN, D. FUCHS and L. LUNDBERG, *J. pharm. Pharmacol. 4, 566 (1952).*
[14] YOSHIHISA KOJIMA and HIROO KUSAKABE, *J. Sci. Res. Inst., Tokyo 50, 193 (1956).* Cited in *Chem. Abstr. 51, 3051 (1957);* B. D. H. Publication Zon *Exchange Resins* (4th Edn.).
[15] S. M. PARTRIDGE and R. G. WESTALL, *Biochem. J. 42, 238 (1948).*

spraying with Dragendorff's reagent. Solvents were all re-distilled before use. Reinecke's reagent was a saturated aqueous solution of ammonium reineckate; chloroplatinic acid reagent was a 10% (w/v) solution of H_2PtCl_6 in 25% (v/v) HCl–ethanol; chloroauric acid reagent was an 8% (w/v) solution of $HAuCl_4$ in 1% (v/v) HCl–water; Bertrand's reagent was a 10% (w/v) solution of silicotungstic acid in water and Knorr's reagent was a saturated solution of picrolonic acid in 95% ethanol. Elemental analyses were done by Messrs P. I. Mowete and F. I. Ozoh of the Department of Chemistry, University of Ibadan.

(2) ***Preparation of Carpaine***

2·5 kg of dried, powdered C. ***papaya*** leaves were processed exactly as described by Govindachari, Pai and Narasimhan.[3] The final product (1.735 g) was a brown gum which failed to yield the expected crystals from acetone. The acetone was distilled off and the residue leached with warm dilute HCl (3 x 25 ml). The acid-soluble matter was treated with Reinecke's reagent until the precipitation of carpaine reineckate became complete. After standing overnight at 5" the reineckate was collected at the pump, washed repeatedly with small amounts of ice-cold water and sucked dry. A small quantity of the complex was recrystallized from aqueous acetone, and afforded glistening, red, translucent, scaly flakes which decomposed slowly without melting from about 185".

The carpaine reineckate was decomposed by dissolving it in 75% (v/v) acetone-water and treating the solution with a slight excess of a saturated aqueous solution of Ag_2SO_4. Silver reineckate was filtered off and the filtrate was concentrated to one-fifth volume, then cooled, treated with excess ammonia and extracted with Et_2O. The dried ethereal extract gave 287 mg of semi-crystalline, chromatographically pure carpaine upon distilling off the solvent and drying the residue ***in vacuo over*** $CaCl_2$. It was recrystallized from acetone, and yielded clusters of short needles (175 mg) m.p. 117-l 19". (Found: C, 69.7; H, 10·6. Calc. for $C_{14}H_{25}NO_2$: C, 70.3; H, 10·5%.)

Carpaine chloroplatinate. *6* mg of carpaine was treated with 2 ml of chloroplatinic acid reagent and the chilled mixture was filtered at the pump. The orange spindle-shaped crystals were washed with cold, dry ethanol and then dried ***in vacuo*** over $CaCl_2$. Yield 12 mg. (Found: C, 36.4; H, 6·0; N, 3·0. Calc. for $(C_{14}H_{25}NO_2)_2.H_2PtCl_6$: C, 36.4; H, 6.1; N, 3·0%.)

Other carpaine complexes. Carpaine did not give satisfactory complexes with other conventional alkaloid precipitants: it failed altogether to give an authentic crystalline picrate from ethanol or ether–benzene.[16] It also did not give a picrolonate by Knorr's method." By the method of Wachsmuth[18] it yielded a silicotungstate as thick white prisms which melted indefinitely with decomposition at 228-296". The reineckate (*vide supra*) decomposed slowly without melting from about 185".

(3) ***Quaternary Buses of C.*** papaya ***Leaves***

3 kg of mature but young leaves of C. ***papaya*** were dried, powdered and extracted for 48 hr in a Soxhlet with $CHCl_3$, in order to separate carpaine from any quaternary bases present. The $CHCl_3$ extract was shaken with dilute HCI (3 x 150 ml). By treating this combined acidic extract with excess ammonia, re-extracting with $CHCl_3$ and distilling the dried $CHCl_3$ extract, 255 mg (0.0085% yield) of chromatographically homogeneous crude carpaine was obtained.

Soxhlet extraction of the leaves was continued with methanol for 48 hr. The methanol was distilled off and the residue was digested with hot $CHCl_3$ (3 x 50 ml) in order to leach out any residual carpaine. Paper chromatographic examination of the filtered $CHCl_3$ digest showed that it contained no more carpaine. The $CHCl_3$-insoluble residue (8.528 g) was examined by paper chromatography. The only basic components detected in it by Dragendorff's reagent were choline (R_f 0.20) together with a trace of another constituent (R_f 0.10) which gave a Dragendorff reaction similar to that of glycinebetaine.[19] 2·65 g of the whole residue was re-dissolved in water. The solution was acidified with dilute HCI and then precipitated with excess Reinecke's reagent. The reineckate complex was collected and decomposed in the usual way with sat. aq. $AgNO_3$. After filtering off the silver reineckate, excess silver ions were precipitated by the addition of HCl. AgCl was filtered off and the final solution (containing quaternary bases together with nitrate and chloride ions) was passed through a column of 100 g of Amberlite IRA-400 resin, in order to remove the ions.[13,14] The column was eluted with 2 l. of CO_2-free, distilled water and the combined effluent was then passed through another column containing 100 g of Amberlite IRC-50 resin, in order to separate choline by virtue of its preferential absorption on this resin. The column was washed further with 1 l. of distilled water before being eluted with 250 ml of 2 N HCI. Evaporation of the acidic eluate at reduced pressure over steam yielded 318 mg of a syrupy residue which was found by paper chromatography to be essentially pure choline hydrochloride. Decolorization of this product with charcoal yielded 244 mg of a white, semi-

[16] H.E. CARTER, P. K. BHATTACHARRYYA, K. R. WEIDMAN and G. FRAENKEL, *Arch. Biochem. Biophys. 38,* 405 *(1952).*

[17] L. KNORR and H. W. BROWNSDEN, *Ber. dtsch. Chem. Ges.* 35, 4470 *(1902).*

[18] H. WACHSMUTH, *J. Pharm. Belge. 6, 86 (1951); Chem. Abs. 45, 9799 (1951).*

[19] H. M. BREGOPF, E. ROBERTS and C. C. DELWICHE, *J. Biol. Chem. 205, 565 (1953).*

crystalline, hygroscopic product-i.e. choline hydrochloride. It was calculated that the total $CHCl_3$ insoluble fraction of the methanol extract (i.e. 8.528 g) should have given altogether 813 mg of choline hydrochloride, which amounts to a yield of choline of 0·02% on the dry weight of the leaves.

The product was identified further as choline hydrochloride by the preparation from it of choline chloroaurate by the method of Carter et *al.*[16] This derivative was obtained as irregular yellow crystals which sublimed slightly from about 220" and melted at 233-234". The m.p. was not changed by admixture with authentic choline chloroaurate. (Found: C, 13.5; H, 3.4. Calc. for $C_5H_{15}O_2N.HAuCl_4$: C, 13.6; H, 3·2%.)

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COMPOSITAE

QUERCETIN-3-(*O*-ACETYL)-β-D-GLUCOPYRANOSID IN *PLUMMERA FLORIBUNDA* UND *HELENIUM HOOPESII*

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Zusammenfassung—Ausser Isoquercitrin und Astragalin wurde Quercetin-3-(*O*-Acetyl)-β-D-Glukopyranosid zum ersten Mal von den methanolischen Extrakten der ***P. floribunda*** und ***H. hoopesii*** isoliert.

Abstract-Besides isoquercitrin and astragalin, quercetin-3-(*O*-acetyl)-β-D-glucopyranosid was isolated for for the first time from the methanolic extracts of ***P. floribunda*** and ***H. hoopesii.***

Pflanzen: (Tribus Helenieae) (1) ***Plummera floribunda*** Gray und *(2)* ***Helenium*** *hoopesii* Gray.

Herkunft: (1) *P. floribunda,* Apache Pass, Cochise County, Arizona, 3 September 1961 (R. S. Barr 61-212),* (2) *H. hoopesii,* Nähe von Gothic, Colorado, August 1960 (B. H. Braun).*

ISOLIERUNG und Identifizierung der Flavonoide : Die oberirdischen Teile der Pflanze wurden zuerst mit Chloroform und dann mit Methanol extrahiert. Nach Abtrennung des Chlorophylls erhielt man die Flavone durch Digerieren der methanolischen Extrakte mit heißem Wasser und Ausschtitteln mit Chloroform, Extraktion mit Äther und Äthylacetat und Chromatographie des Mischkristallisates an Zellulosesaulen mit 10 %iger Essigslure. Die Identifizierung erfolgte durch DC-Co-Chromatographie mit Testsubstanzen in den Systemen: (a) 10% Essigsäure Zellulose und (b) BEW (4: 1: 5) Zellulose, (c) ÄA–AS–Wa (10:2: 3) Kieselgel, bzw. durch Nachweis der Spaltprodukte nach saurer und alkalischer Hydrolyse, sowie durch *W* und NMR-Spektroskopie.

* Belegexemplar hinterlegt im Herbarium der Florida State University.