Preliminary Phytochemical Investigation of *Maytenus trichotomus* (*Celastraceae*)

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The following compounds were identified and separated from *Maytenus trichotomus* by means of paper, thin-layer, and column chromatographic techniques: caffeine, caffeic acid, chlorogenic acid, ferulic acid, and benzoic acid. A leucoanthocyanin (leucocyanidin) was also isolated.

MAYTENUS TRICHOTOMUS[1] has been the subject of a preliminary investigation to determine whether any of its purported pharmacological properties are valid. The plant has been and still is used by Mexican natives as an anti-infective in febrile-type diseases and also as an antitumor agent. In a routine screen of plants for antitumor properties carried on by Caldwell of these laboratories, the plant has exhibited some antitumor activity. A subsequent report on the nature of the antitumor substances will be forthcoming.

The literature does not mention chemical constituents of the *Trichotomus* species. There have been several reports which deal with seed contents, mucilage residues (1–8), and similar information. Stellfeld (9) reported analgesic and diuretic properties in *M. ilicifolia*. Pereira (10, 11) reported caffeine, chlorogenic acid, and other phenolic compounds in the same plant.

EXPERIMENTAL

Extraction and Preliminary Tests.—Ten kilograms of *M. trichotomus*, collected in Vera Cruz, Mex., was air-dried, ground, and extracted in a Lloyd extractor with petroleum ether (30–60°) and ether U.S.P. until essentially complete extraction was accomplished. The marc then was subjected to an acetone extraction again using the Lloyd extractor. This extract then was evaporated under reduced pressure to a 300-Gm. brown mass.

A portion of the brown mass was dissolved in a 50% aqueous alcoholic solution. This solution gives a positive test with Mayer's and Tanret's reagents, indicating the possible presence of alkaloids.

Another portion of this solution was placed on Whatman No. 1 paper and subjected to paper chromatography using a solvent system of 20% aqueous acetic acid. Some visible yellow spots and some bright blue spots under ultraviolet light were detected. Spraying the paper with 30% aqueous chlorosulfonic acid solution resulted in the appearance of a pink spot. As a result of the above color reaction, the possibility of leucoanthocyanins and flavones was considered.

Caffeine and Related Acids.—Ten grams of the acetone extract (brown mass) indicated above were extracted with 600 ml. of 5% methanolic ether. Upon evaporation of the ether, 3 Gm. of a green mass was obtained. Fifty milligrams of this mass was dissolved in alcohol and subjected to a series of paper and thin-layer chromatographic experiments, the results of which were compared with known compounds. Using seven different solvent systems, the presence of ferulic, caffeic, and chlorogenic acid (Table I) and caffeine (Table II) was demonstrated. In the paper chromatographic experiments, 11 × 46 cm. Whatman No. 1 paper was used. In the thin-layer work, Silica Gel G was the substrate and the plates were 4 × 20 cm. The developing color reagent used in Table II was 0.2% fluorescein in methanol.

The remainder of the 3-Gm. green mass was dissolved in 250 ml. of ether U.S.P., and then extracted with 5% aqueous sodium bicarbonate. The ether-soluble portion was washed with water which was added to the sodium bicarbonate solution. Upon evaporation to dryness of the remaining ether solution, a yield of 500 mg. of a crude mixture containing caffeine was obtained.

Modification of the thin-layer chromatographic technique by placing a larger amount of Silica Gel G on larger plates (35 Gm./50 ml. of water, 20 × 20 cm. plates) resulted in the ability to separate and isolate larger quantities of materials. One hundred milligrams of the crude caffeine-containing mixture was applied to two plates and using solvent system 5, Table II, 11.2 mg. of caffeine was obtained. The infrared spectrum of this isolated material was identical with that of a spectrum of known caffeine. Spectra were run on a Perkin-Elmer Infracord, model 137. The sodium bicarbonate solution was acidified with concentrated hydrochloric acid and extracted with 300 ml. of ether. The ether was evaporated to dryness and 520 mg. of a crude mixture of acids was obtained. The mixture of acids was dissolved in boiling water and cooled; 183 mg. of material settled out. Recrystallization of this acid from boiling water gave a melting point of 122°. The infrared spectrum and mixed melting point results indicated the compound to be benzoic acid. The water solution was then evaporated to a small volume and a mixture of three acids, ferulic, caffeic, and chlorogenic, separated. Utilizing the modified thin-layer procedure discussed above and

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[1] Identification confirmed by Robert Barr, Research Associate, and Dr. Charles Mason, Curator of the Herbarium, Botany Department, The University of Arizona. A reference specimen was also deposited.

TABLE I.—PAPER AND THIN-LAYER CHROMATOGRAPHIC COMPARISONS OF KNOWN AND ISOLATED FERULIC, CAFFEIC, AND CHLOROGENIC ACIDS

No.	Solvent System	Method	D.T.[c]	Ferulic Acid		Caffeic Acid		Chlorogenic Acid	
				Unknown	Std.	Unknown	Std.	Unknown	Std.
1	20% AcOH	PC[a]	10	0.79	0.80	0.69	0.70	0.91	0.90
2	Gl. AcOH–c. HCl–H_2O (5:1:5)	PC	10	0.83	0.81	0.70	0.71	0.93	0.95
3	(Upper phase) *n*-BuOH–H_2O–formic acid (4:1:1)	PC	10	0.92	0.90	0.85	0.85	0.85	0.85
4	*n*-BuOH–saturated with 1.5 *N* NH_3	PC	10	0.11	0.12	0.09	0.09	0.54	0.53
5	(Upper phase) Benzene–Gl. AcOH–H_2O (6:7:3)	PC	5	0.71	0.69	0.15	0.16	0.15	0.16
6	Benzene–dioxane–Gl. AcOH (90:25:4)	TLC[b]	1	0.75	0.76	0.49	0.49	0.085	0.084
7	Benzene–MeOH–Gl. AcOH (45:8:4)	TLC	1	0.91	0.90	0.82	0.83	0.49	0.48

[a] Paper chromatography. [b] Thin-layer chromatography. [c] Development time (hr.).

TABLE II.—PAPER AND THIN-LAYER CHROMATOGRAPHY OF KNOWN AND ISOLATED CAFFEINE

No.	Solvent System	Method	D.T.	Unknown	Std.
1	Benzene–dioxane–AcOH (90:25:4)	TLC	1	0.37	0.36
2	Benzene–MeOH–AcOH (45:8:4)	TLC	1	0.62	0.64
3	$CHCl_3$–EtOH (95:5)	TLC	2	0.08	0.07
4	(Upper phase) benzene–AcOH–H_2O (4:4:2)	PC	5	0.82	0.83
5	Benzene–ether–AcOH–MeOH (120:60:18:1)	PC	5	0.86	0.84
6	MeOH–acetone–NH_3 (1:1:0.03)	PC	8	0.73	0.72
7	Benzene–MeOH (95:5)	PC	5	0.81	0.83

TABLE III.—PAPER AND THIN-LAYER CHROMATOGRAPHY OF KNOWN ANTHOCYANIDIN AND ISOLATED CYANIDIN

No.	Solvent System	Method	D.T.	Unknown	Std.
1	Water–AcOH–c. HCl (10:30:3)	PC	16	0.5	0.5
2	(Upper phase) *n*-BuOH–2 *N* HCl (1:1)	PC	16	0.66	0.67
3	88% Formic acid–3*N* HCl (1:1)	PC	16	0.25	0.24
4	(Upper phase) *n*-BuOH–AcOH–H_2O (4:1:5)	PC	16	0.61	0.62
5	*n*-BuOH–AcOH–c.HCl (5:3:1)	TLC	2	0.88	0.89

TABLE IV.—PAPER CHROMATOGRAPHY OF THE HYDROLYZED PRODUCTS OF ISOLATED CYANIDIN AND KNOWN COMPOUNDS

No.	Solvent System	Method	D.T.	Protocatechuic A.		Phloroglucinol	
				Unknown	Std.	Unknown	Std.
1	(Upper phase) *n*-BuOH–AcOH–H_2O (5:1:5)	PC	10	0.77	0.75	0.64	0.64
2	20% AcOH	PC	10	0.64	0.64	0.72	0.72

TABLE V.—PAPER CHROMATOGRAPHY OF SUGARS OBTAINED FROM HYDROLYSIS OF LEUCOANTHOCYANIN COMPARED WITH KNOWN SAMPLES

No.	Solvent System	Method	D.T.	D-Glucose		L-Rhamnose	
				Unknown	Std.	Unknown	Std.
1	(Upper phase) *n*-BuOH–AcOH–H_2O (5:1:5)	PC	10	0.15	0.15	0.41	0.42
2	Phenol–1% NH_3	PC	16	0.38	0.39	0.66	0.66

solvent system 7, Table I, a separation of the three acids was obtained. The yields from 34 mg. of mixture were 3.9, 3.2, and 3.8 mg., respectively.

Infrared spectrum of the isolated material was again compared with known materials as further proof. They were identical.

A series of column chromatographic methods was employed to separate the caffeine from the colored pigments in the brown mass preliminary extract. The most effective separation was obtained by means of a column (40 × 5 Gm.) packed with Cellex E (an anion exchange cellulose powder, Calbio Chemical Co., Los Angeles, Calif.). The column was packed with a mixture of 300 Gm. of Cellex E in 50% aqueous alcohol. The colored materials were adsorbed by the column. The column was first eluted with alcohol (500 ml.). A pale yellow solution was obtained. Further elutions with water (500 ml.) and with 10% aqueous sodium hydroxide, in an attempt to remove the pigments from the column, were not successful. The pale yellow solution was evaporated under reduced pressure to a small volume and submitted to paper chromatographic analysis using the same systems indicated above. This again demonstrated the presence of caffeine separated from the colored pigments.

Precipitation and Conversion of Leucoanthocyanin to Anthocyanin and Subsequent Hydrolysis.—The remaining portion of the 10-Gm. sample of the acetone extract which was extracted with 5% methanolic ether was dissolved in 100 ml. of 95% ethanol, and upon the addition of 750 ml. of ether, a precipitation of crude leucoanthocyanins occurred.

One gram of the precipitate was separated, dissolved in 50% aqueous ethanol, and boiled with 20% hydrochloric acid for 1 hr. A bright red color appeared. After the addition of 100 ml. of water, the solution was extracted with 5 × 20 ml. of isoamyl alcohol. The isoamyl alcohol extract was evaporated to dryness to obtain 531 mg. of a red powder.

This material was dissolved in methanol and subjected to paper and thin-layer chromatography. The results in Table III indicate the probable presence of cyanidin as well as some other brown streaking material. Using a quantitative paper chromatographic analysis technique, 500 mg. of the crude anthocyanin mixture was dissolved in 10 ml. of alcohol. A 20 × 46 cm. Whatman No. 3 paper was spotted with precisely 10 mg. of the above mixture using a micropipet. Solvent system *1*, Table II, was used. The material from ten papers, at R_f value 0.5, was cut from the paper and extracted with 95% ethanolic 0.1% hydrochloric acid. The yield of cyanidin was 22.5 mg. A 2×10^{-5} *M* solution of the compound in 95% ethanolic 0.1% hydrochloric acid then was placed in a Beckman ultraviolet spectrophotometer model DB. The spectrum showed absorption peaks at 540, 272, and 212 mμ. Comparison with a known sample of cyanidin obtained from K & K Laboratories, Plainview, N. Y., was made and the resultant curves were the same.

Hydrolysis of the Anthocyanin.—The 22.5 mg. of cyanidin isolated above was boiled with 6 *N* H_2SO_4 for 72 hr. The acid and phenolic portions were separated in a similar manner as described under *Caffeine and Related Acids*. Utilizing paper chromatography (Whatman No. 1 paper) and comparison with known compounds, the presence of protocatechuic acid and phlorglucinol was shown (Table IV). The color reagent was 5% ferric chloride solution. (The solution that was hydrolyzed with sulfuric acid should contain phloroglucinol and protocatechuic acid. The solution was extracted with ether. This extraction removes both the acid and the phenolic fractions. The ether extract is next treated with 5% sodium bicarbonate solution. The aqueous phase contains the sodium salt of the acid, and the ether portion contains the phenol. The sodium bicarbonate solution is made just acidic with hydrochloric acid and extracted with ether. Upon evaporation of the ether, protocatechuic acid is obtained. The original ether solution remaining from the sodium bicarbonate solution extraction is evaporated to yield the phenol phloroglucinol.)

A portion of the crude leucoanthocyanin precipitate obtained by the addition of ether to the acetone extract was dissolved in 50% ethanol and then boiled with 0.1 *N* HCl for 24 hr. After washing with isoamyl alcohol until colorless, and concentration of the aqueous part to a small volume, paper chromatography was again used showing the presence of D-glucose and L-rhamnose (Table V). The detecting reagent was a 0.1 *M* solution of aniline phthalate in *n*-butanol.

SUMMARY

The following compounds were isolated and identified from *M. trichotomus*, utilizing thin-layer, paper, and column chromatography as well as spectrophotometric techniques: caffeine, caffeic acid, ferulic acid, chlorogenic acid, benzoic acid, and leucocyanidin. The sugars associated with the leucocyanidin were shown to be D-glucose and L-rhamnose.

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