

Chemical Investigation of Hard Wood Extractives-II

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ABSTRACT

The present work reports the chemical investigation of extractives of the hardwood Bhirra (*Chloroxylon swietenia*). Extraction was carried out as reported in Part-I^{1a}. Three chemical constituents could be identified as condensed tannin (Polyphenol), rosin ester (wood resin) and alkaloid.

Auld² and Mookerji³ have reported the presence of alkaloid and resin (non-basic) crystalline constituent in Bhirra-bark without any experimental details while studying this species from pharmacological study point of view. It is, therefore, proposed to study the extraneous components of Bhirra-wood systematically by following the scheme of Carlberge and Kurth¹ with slight modification.

EXPERIMENTAL

Proximate analysis of Bhirra-wood on even dry basis carried out as per TAPPI Standards are tabulated in Table-I.

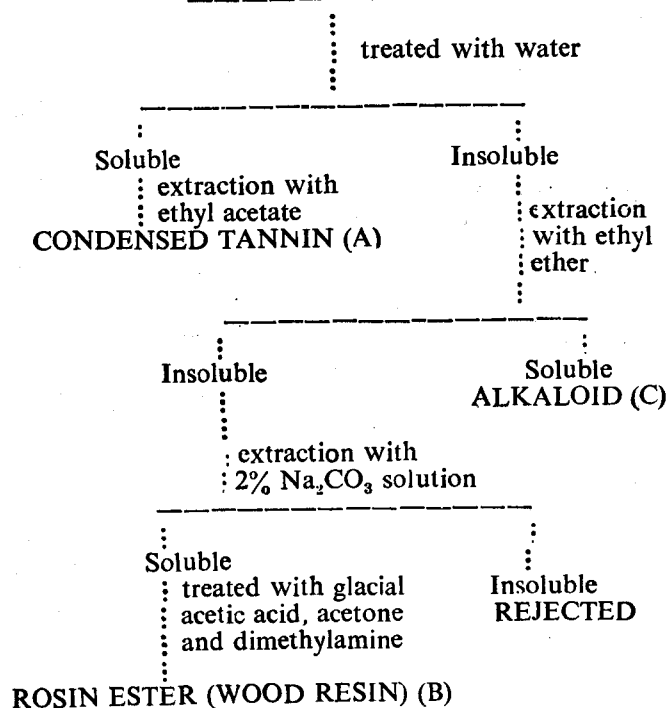
TABLE-I
PROXIMATE ANALYSIS OF BHIRRA
(*Chloroxylon swietenia*)

S. No.	Determination	Percentage
1.	Ash	1.40
2.	Solubility in—	
	(i) Cold water	4.80
	(ii) Hot water	3.10
	(iii) Ether	0.20
	(iv) One per cent Sodium hydroxide	18.50
	(v) Alcohol-benzene (1:2 v/v)	3.20
3.	Lignin (72% Sulphuric acid method)	26.50
4.	Holo cellulose (By Ritter and Kurth method)	65.70
5.	Pentosan	14.70

EXTRACTION WITH ETHANOL-BENZENE MIXTURE

Bhirra-wood sample was extracted with ethanol-benzene (1:2 v/v) mixture for 24 hrs. with occasional stirring in a Soxhlet apparatus. After removal of solvents the the extract was treated as per scheme-I for the separation of various components.

SCHEME—I Ethanol-Benzene Extract



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ISOLATION AND IDENTIFICATION OF WATER SOLUBLE COMPOUND (A)

To isolate this compound, ethanol-benzene extract was treated repeatedly with water, the aqueous layer was removed, concentrated and extracted with ethylacetate, saturated with sodium chloride, again extracted with ethyl acetate and dried over sodium sulphate. Dried ethylacetate extract was further concentrated and was added to excess warm hexane when tannin was precipitated⁴ as amorphous powder; this was washed with hexane dried m.p. 215°C. This compound, m.p. 215°C gave green colour with alcoholic ferric chloride⁵ which changes to red on addition of sodium chloride and intense violet colour with methanol-sulphuric acid solution⁶. It also gave precipitation with lead-acetate solution, which was soluble in acetic acid¹¹. These tests are standard tests for identification of tannins.

The compound (A) was resolved over silica gel-G by n-butyl alcohol-acetic acid-water (4:1:5 v/v) as the developing solvent^{7,8}. The compound was detected by spraying first with aqueous ferric chloride (2%) and then it was kept in contact with strong ammonia vapours in chromatogram. The grey-blue spot appeared by examination under ultra-violet lamp; R_f 0.75^{7,8}.

These properties correspond very closely to those of condensed tannins such as catechin or galocatechin.

ISOLATION AND IDENTIFICATION OF ETHER INSOLUBLE COMPOUND (B)

In order to remove this as completely as possible, the whole water insoluble material left after the removal of compound (A) was shaken several times with cold ethyl ether. The combined ethyl ether solvent was filtered and preserved for the isolation of the compound (C).

The compound (B) was shaken with benzene-ethanol mixture and was extracted with 5% Na_2CO_3 solution. The insoluble sodium salt was rejected. The compound (B) was obtained in the form of amine salt by treating soluble aqueous sodium salt portion successively with glacial acetic acid, acetone and a solution of diamylamine⁹, slowly with vigorous shaking. The amine salt of rosin ester thus obtained was crystallized from acetone m.p. 110°C.

Crystalline compound (B) was easily soluble in alcohol, glacial acetic acid, benzene and chloroform less soluble in ethyl ether and practically insoluble in water and sodium bicarbonate¹⁰. Thus showing the presence of rosin esters/sterols. It dissolves in conc. H_2SO_4 acid with the formation of a dark brown colour. Its solution in conc. HNO_3 acid was orange and in conc. HCl acid, it gave a yellow colour. Furthermore, it decolourised potassium permanganate solution and Br_2 water indicating the unsaturated nature of the compound.

The compound (B) was dissolved in 95% hot alcohol and treated with 1% hot alcoholic solution of digitonin. No white precipitate was obtained indicating that crystalline compound is of rosin ester—a type of wood resin. This view was further confirmed by the fact that it was not saponified and gave the colour reaction such as Libermann-Burchard¹¹ and Cohen reaction¹¹. The identification of rosin ester was also confirmed by the formation of red to violet colour by adding this compound to a mixture of benzene, acetic anhydride¹¹ and one drop of conc. H_2SO_4 , shaken for 15 sec.

The compound (B) was resolved over silica gel-G by cyclohexane-ethylacetate as the developing solvent¹². The compound was detected by spraying with the phosphomolybdic acid; R_f 0.085¹².

The above studied properties correspond very closely to those of rosin ester and suggestive of sterol type compound.

ISOLATION AND IDENTIFICATION OF ETHER SOLUBLE COMPOUND (C)

As the ether extract was being concentrated on water bath to about 2-3 cc a dark yellowish viscous matter, having a very bitter smell began to separate out. This was further digested with 0.1% HCl acid when the base completely passed into acid layer as tested with Dragendorff's reagent. The acid digest was cooled in ice and carefully basified with aq. sodium carbonate. The base separating in flocks was vigorously shaken with ether when the base passes into the ether layer. The pale yellow ether extract was twice washed with water, dried over anhydrous sodium sulphate and distilled. The residue was an oil which slowly solidified to a crystalline mass. This was successively crystallized from ethylacetate, alcohol and acetone in glistening colourless prisms. m.p. 170°C.

Its solution in alcohol was neutral to litmus. Being a weak base, it did not form stable salt with mineral acids and could be quantitatively precipitated from its acid solution by sodium carbonate. Its alcoholic solution did not produce any colouration with ferric chloride indicating the basic nature of alkaloid.

The compound gave pale yellow solution with a green fluorescence with conc. H_2SO_4 , orange red changing to red with conc. HNO_3 , pale yellow changing to olive green after a day with Froehde's reagent, and pale yellow changing to red, finally to orange red with Erdman's reagent showing alkaloid nature^{2,3} of the compound.

The base gave an orange precipitate with bismuth iodide, a white precipitate with Meyer's reagent, a chocolate brown precipitate with a solution of iodine in KI and crystalline yellow precipitate with picric acid.

The base was readily soluble in acetone, fairly in chloroform, sparingly in ethyl acetate, benzene and ether and insoluble in pet. ether and water. The base readily dissolved in aqueous HCL, HNO_3 and H_2SO_4 acids, but the corresponding salts could not be isolated in the pure state.

Qualitative analysis for elements showed the presence of N, C, H, O. Analysed and observed C 64.50%, H 5.30%, N 4.50%. Calculated for $C_{11}H_{13}O_4$, $N^{2.3}$: C 65.06%, H 4.85%, N 5.31% m.p. 175°C.

It afforded a picrate derivative, crystallised from alcohol m.p. 195–195° decomp.

The base was resolved over silica gel-G plate by chloroform-methanol-diethylamine (80 : 20 : 1) as the developing solvent¹³. The compound was detected by spraying with solution A (bismuth nitrate 0.85 g, water 100 ml, and glacial acetic acid 10 ml), and solution B (potassium iodide 8 g and water 100 ml) mixed with glacial acetic acid and water. Well defined red spot on a pale orange background was visible under an u.v. lamp; R_f 0.75⁸.

From all these properties and the similar observation made earlier, the compound (C) appeared to be identical with Skimmianine and chloroxytonine³ which are quinoline alkaloids.

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