RHAPONTICIN, AND ANTHRAQUINONE DERIVATIVES FROM *RHEUM EMODI*, WALL. (INDIAN, OR HIMALAYAN RHUBARB).

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The Chinese rhubarb of commerce, which is official in the Pharmacopoeias of many European countries and the United States of America, is either *Rheum palmatum,* Linn., *Rheum officinale,* Baill., or "other species of *Rheum* grown in China and Tibet", and is one of the most popular cathartic drugs. It generally comes to the European market from China and is sometimes found adulterated with less active varieties of *Rheum,* e.g., *Rheum rhaponticum,* Linn., which are found in Austria and adjoining countries ; in recent years, however, the official rhubarb has been grown in England, France and other European countries. Since it is generally known that the glucosides of hydroxyanthraquinones are at least partially responsible for the cathartic action of rhubarb, its chemistry, mainly with respect to the nature of the anthraquinone derivatives, has engaged the attention of many investigators.

Although the earliest inquiries date from the first quarter of the last century, it is only since the beginning of this century that systematic work on the drug has been conducted. Hesse (*Annalen,* 1899, **309,** 32) found chrysophanic acid and its methyl ether, emodin, rhabarberon (an isomer of emodin), rhein, and some other compounds in a sample of Chinese rhubarb (*Rheum officinale,* Baill.) and chrysophanic acid and rhaponticin in Austrian (*Rheum rhaponticum,* Linn.) and English rhubarbs. The sample of English rhubarb, however, was shown by the same author (*J. pr. Chem.,* 1908, **77,** 32) to be *Rheum rhaponticum,* Linn. and not *Rheum palmatum,* Linn. as originally supposed. In this connection he also described the analysis of Austrian rhubarb which was found to contain rhaponticin, anhydro-rhapontigenin, $C_{15}H_{12}O_3$, iso-rhapontigenin, $C_{15}H_{14}O_4$, chrysophanic acid, $C_{15}H_{10}O_4$, and two other isomers of emodin, $C_{15}H_{10}O_5$, with gallic acid, rhapontic acid, $C_{17}H_{16}O_6$, and glucosides of anthraquinone derivatives. The methyl ether of chrysophanic acid has been shown indirectly by Oesterle and Johann (*Arch. Pharm.,* 1910, **248,** 476) to be impure chrysophanic acid mixed with emodin monomethyl ether which is usually associated with the former in rhubarb.

Tschirch and Heuberger (*Arch. Pharm.*, 1902, **240**, 596) isolated from a sample of Chinese rhubarb, presumably *Rheum officinale*, Baill., chrysophanic acid, emodin, rhein and a mixture of tannin- and hydroxyanthraquinone-glucosides. Tschirch and Cristofoletti (*Arch. Pharm.*, 1905, **243**, 443) found in Austrian rhubarb rhaponticin, chrysophanic acid and its methyl ether (*cf.* Oesterle and Johann, *loc. cit.*), chrysopontin, $C_{16}H_{16}O_5$, corresponding in composition to methoxy-dihydroxymethyltetrahydroanthraquinone, and a mixture of hydroxyanthraquinone-glucosides. They could not isolate either emodin or rhein. Tschirch and Edner (*Arch. Pharm.*, 1907, **245**, 139) isolated from English rhubarb (*Rheum officinale*, Baill.) only chrysophanic acid, emodin, iso-emodin (rhabarberon of Hesse, *loc. cit.*) without rhaponticin; in French rhubarb (*Rheum rhaponticum*, Linn.) they found rhaponticin, chrysophanic acid and chrysopontin.

Tutin and Clewer (*J.C.S.*, 1911, **99**, 946) found in a sample of "Shensi" (Chinese) rhubarb, cinnamic and gallic acids, rhein, emodin and its monomethyl ether, chrysophanic acid, aloe-emodin, rheinolic acid, a crystalline mixture of the glucosides of most of the above hydroxyanthraquinones, together with a non-glucosidic resin which, on hydrolysis with alkali, gave small amounts of cinnamic acid, emodin, aloe-emodin and gallic acid. They found this last non-glucosidic resin to be physiologically the most active constituent.

Tschirch and Ruszkowski (*Arch. Pharm.*, 1913, **251**, 121) found in a Russian variety of rhubarb (*Rheum rhaponticum*, Linn.) rhaponticin, emodin and its monomethyl ether, chrysophanic acid with tannin- and hyroxyanthraquinone-glucosides. Semmel (*Arch. Pharm.*, 1918, **256**, 91) found "Shensi" rhubarb (*Rheum palmatum*, Linn.) to contain chrysophanic acid, aloe-emodin, rhein, and hydroxyanthraquinone-glucosides. Holmström (*Schweiz. Apoth. Ztg.*, 1921, **59**, 169 and 183; through *Chem. Abs.*, 1921, **15**, 2427) during his analysis of *Rheum emodi*, Webb. (probably from Switzerland), found rhaponticin, chrysophanic acid, emodin (?) and a compound similar to rheochrysin.

Rheum emodi, Wall. belonging to the natural order, *Polygonaceae*, is one of the Indian rhubarb species found wild in Nepal, Kashmir, Sikkim and Bhutan at altitudes of eleven to twelve thousand feet. The Central Indigenous Drugs Committee (which functioned only between the years 1901 and 1916) appointed by the Government of India to make a clinical study of indigenous drugs the properties of which were already known, subjected this drug to a detailed clinical test. The Committee assumed that it contained only the anthraquinone derivatives usually found in official rhubarb, and found (Second Report, 1909, pp. 71-74 and Third Report, 1916, pp. 1-22) that the powdered drug caused

griping pain in a large number of cases ; the general opinion was that it was unsatisfactory as a purgative.

Griping action is not usually associated with preparations from official rhubarb and since no chemical examination of Indian or Himalayan rhubarb has been made hitherto, it seemed desirable to seek definite information regarding the constituents responsible for the griping effect, and to survey the anthraquinone derivatives responsible for its cathartic action.

EXPERIMENTAL.

The material employed consisted of dry and powdered rhizomes obtained from Kashmir forests.

Preliminary examination for alkaloids.—Digestion with Prollius's fluid for four hours gave negative results.

Starch, other carbohydrates and tannins.—The filtrate from thorough digestion with warm water for about half an hour showed the presence of starch, tannins, and a reducing sugar.

Steam distillation.—About one litre of acidic distillate from 500 g. was thoroughly extracted with ether, and this after washing with sodium carbonate solution followed by sodium hydroxide left on distillation a small amount of pale yellow oil having the characteristic odour of the drug.

Extraction with solvents.—From 100 g. extracted in a Soxhlet apparatus successively with various solvents the following percentages of extracts dried at 100° were obtained :—Petroleum ether (b.p. 50-60°), 2.0 ; ethyl ether, 1.9 ; chloroform, 1.3 ; ethyl acetate, 15.8 ; acetone, 9.5 and ethyl alcohol (91 per cent.) 12.5 : total, 43.0. Excepting the last two, the extracts consisted of yellow crystalline material. For a detailed examination, the powdered rhizomes (32 kg.) were thoroughly extracted with alcohol (91 per cent.) which was then removed under reduced pressure, the viscous extract being freed from essential oil by steam-distillation. The yellow distillate was extracted several times with ethyl ether, which on subsequent extraction with ammonium and sodium carbonate solutions yielded a very small amount of black material. The sodium hydroxide extract gave a small quantity of chrysophanic acid, m.p. 190-191°.

The ether, after extraction with alkalis, left as residue (25 g.) an orange essential oil which distilled for the most part at 55-60°/10 mm., and a very small portion at 140-145° ; both fractions had in a very

high degree the characteristic smell of the drug. The first fraction was unfortunately lost.

Non-volatile constituents of the extract.

The dark aqueous residue from steam-distillation was decanted and filtered from the brown resinous mass (B), which was washed with water and the washings added to the aqueous filtrate (A), amounting to about 60 litres. Extracts of this with ethyl ether (A1), ethyl acetate (A2) and amyl alcohol (A3), along with the aqueous residue (A4) were examined separately.

Extract (A1) : *Isolation of Rhein,* $C_{15}H_8O_6$.—After removing ether the crystalline residue was recrystallised from amyl alcohol, and then from ethyl alcohol four times, when orange, glistening needles melting at 320-321° were obtained. The acetyl derivative was greenish yellow, melting at 256-257°. Analysis confirmed the above empirical formula for rhein.

Isolation of Emodin, $C_{15}H_{10}O_5$.—The amyl alcoholic filtrate from crude rhein was extracted with 10 per cent. sodium carbonate solution which, on acidification, yielded a reddish brown precipitate (about 15 g.). This, after five crystallisations from ethyl alcohol and finally from pyridine was orange yellow, partially subliming at about 230° and melting to a dark red liquid at 254-255°. The bright yellow acetyl derivative melted at 197-198° and analysis confirmed the formula above (Found : C, 66.8 ; H, 4.1 $C_{15}H_{10}O_5$ requires C, 66.6 ; H, 3.7, per cent.).

The combined mother liquors were concentrated and poured into ethyl ether when some black material settled. The ethereal filtrate was extracted with 10 per cent. ammonium carbonate solution which, on acidification, gave only a dark red viscous product from which no crystalline compound corresponding to rheinolic acid of Tutin and Clewer (*loc. cit.*) could be obtained. After extracting with sodium carbonate, the amyl alcohol solution was extracted with 10 per cent. sodium hyroxide, acidification yielding a product which, when crystallised twice from chloroform, was orange yellow melting at 226-227°. This gave an acetyl derivative which softened at 182° and completely melted at 187°. The amount of the material isolated in a pure form being very small, it could not be further examined.

Isolation of Chrysophanic acid, $C_{15}H_{10}O_4$.—The combined chloroform mother liquors were then extracted first with 0.5 per cent. sodium hydroxide solution (to remove the above compound) and then with 5 per cent. sodium hydroxide ; this on acidification gave a yellow product which after six crystallisations from alcohol formed golden

yellow, shining plates melting at 194-195°. The acetyl derivative melted at 204°, and analysis confirmed the formula of chrysophanic acid.

Extract (A2): *Isolation of Rhaponticin,* $C_{21}H_{24}O_9$.—The concentrated extract deposited about 200 g. of pale yellow crystals. After being washed once with 20 per cent. ethyl alcohol and recrystallised five times from 5 per cent. alcohol with norit carbon, colourless prismatic crystals melting at 233-234° were obtained. Analysis agreed with the formula $C_{21}H_{24}O_9$, while hydrolysis with dilute sulphuric acid gave glucose and rhapontigenin which, when crystallised thrice from methyl alcohol, yielded colourless glistening needles melting at 184-185°.

Glucoside of chrysophanic acid.—The ethyl acetate filtrate and washings after separating crude rhaponticin were further concentrated to small volume when a brown pulpy mass floated, and was crystallised from 91 per cent. alcohol. The reddish brown product melted at 204-205° and on hydrolysis gave chrysophanic acid (m.p. 192-193°) with a reducing sugar in quantity too small for identification.

Extract (A3): *Rhaponticin and mixed glucosides.*—After removing the solvent under reduced pressure and digesting the residue with 5 per cent. alcohol, the pale yellow product (about 150 g.) was crystallised several times from 20 per cent. alcohol and became colourless; melting at 232-233° it was found to be rhaponticin.

The combined alcoholic filtrates after separating rhaponticin were concentrated to small volume, but the brown amorphous precipitate could not be crystallised. It was therefore hydrolysed with dilute sulphuric acid, giving a reducing sugar and a mixture of anthraquinone derivatives consisting mostly of emodin and chrysophanic acid.

Aqueous residue (A4).—A portion was treated first with neutral lead acetate solution and the filtrate from the heavy precipitate further treated with basic lead acetate solution which gave only a small precipitate. The solids were combined, de-leaded with hydrogen sulphide, and the filtrate from lead sulphide extracted first with ethyl ether and then with amyl alcohol. The ether extract gave a small amount of a brown residue which was strongly acidic to litmus, while the amyl alcoholic extract after removal of solvent under reduced pressure gave a reddish brown residue consisting of mixed glucosides resembling those obtained from A3.

The filtrate from lead precipitate having been de-leaded and decolourised with norit carbon, was concentrated under reduced pressure. The yellow, syrupy residue contained a considerable amount of glucose,

but did not contain any di- or poly-saccharide, the amount of reducing sugar being found unchanged by attempted hydrolysis.

Water-insoluble, brown, resinous mass (B).—The brown viscous mass was well mixed with about 5 kg. of the alcohol-extracted drug, dried completely and extracted in a specially devised continuous extraction apparatus successively with low-boiling petroleum ether (B1), ethyl ether (B2), chloroform (B3) and ethyl acetate (B4). Preliminary work having shown that the acetone and alcoholic extracts gave only a brown, amorphous, tarry material, these extractions were omitted.

Extract (B1).—The golden yellow, crystalline material deposited on concentration was washed with the solvent and crystallised three times from a mixture of chloroform and ethyl acetate (2 :1), when chrysophanic acid (about 20 g.) melting at 193-194° was obtained. The combined filtrates and washings after separating the chrysophanic acid were concentrated, the residue dissolved in ethyl ether and extracted successively with 5 per cent. sodium carbonate and hydroxide solutions to remove emodin and chrysophanic acid, respectively. After evaporating ether the viscous residue was saponified with alcoholic sodium hydroxide solution, the alcohol then distilled, and the residual, strongly alkaline solution, mixed with purified filter-paper pulp, dried and extracted with dry ethyl ether in a Soxhlet apparatus. The unsaponifiable material thus removed, after crystallisation four times from 91 per cent. alcohol, was colourless and melted at 135-136°, giving the common colour reactions of phytosterols and yielding an acetyl derivative melting at 120-121°. From the combined mother liquors, the remaining phytosterol was removed by treating with a hot solution of digitonin, the filtrate from which contained a brown, waxy material.

The Fatty Acids.

After removal of unsaponifiable matter, the soap was extracted from the filter-paper pulp with hot water, and the acids separated as usual into saturated and unsaturated acids by Twitchell's method (*Ind. Eng. Chem.*, 1924, **13,** 806).

Saturated acids.—The methyl esters were prepared in the usual manner and distilled under 2 mm. in three fractions, the acids corresponding to each fraction being crystallised from alcohol. The first (2 g.) on three crystallisations gave a small amount of a product having m.p. 57-58° and M.W. 270, probably a mixture of palmitic and stearic acids. The second (2 g.) and third (0.5 g.) fractions were combined and on being crystallised four times yielded an acid of m.p. 77-78° and M.W. 425. The combustion values (Found : C, 79.6 ; H, 14.1) for this acid however do not agree with those of any of the known acids.

The melting point corresponds to that of cerotic acid while the molecular weight agrees nearly with that of montanic acid. The amount was too small for confirmatory work.

Unsaturated acids.—The methyl esters were distilled under 2 mm. in three fractions from which the yellow colour of some anthraquinone derivatives was removed by washing with cold dilute sodium hydroxide. The first fraction was too small for investigation. The second and third fractions boiled at almost the same temperature, so were combined, hydrolysed and oxidised with cold permanganate solution. The hydroxy-acids thus obtained were extracted in a Soxhlet apparatus successively with petroleum ether, ethyl ether and ethyl alcohol. The petroleum ether yielded only a small amount of a viscous material. The ether extract, on being crystallised twice from ethyl alcohol, gave colourless crystals having m.p. 128-130° and M.W. 318, evidently dihydroxystearic acid. The alcoholic extract on further crystallisation was colourless, having m.p. 165-166° and M.W. 347; this molecular weight agrees well with that of a tetrahydroxystearic acid, but the melting point is lower than that of sativic acid. A lower melting point for tetrahydroxystearic acid has been observed before by several investigators, and it is therefore possible that this product is a mixture of isomeric acids having the formula, $C_{18}H_{36}O_6$.

The Extracts, B_2, B_3 *and* B_4.

Extract (B2).—Concentration gave a dark red solution with a large proportion of yellow solid which was thoroughly washed with ether and crystallised six times from hot alcohol; the lustrous, golden yellow plates melting at 194-195° consisted of chrysophanic acid. The residue from the combined alcoholic mother liquors was crystallised several times from pyridine; the deep orange yellow needles melting at 254-255° consisted of emodin.

Extract (B3).—The brown viscous residue after removing the solvent was washed with cold alcohol several times, the washings on concentration depositing a small amount of emodin. The alcohol-insoluble residue was digested with cold sodium carbonate solution and filtered, acidification giving a yellow, crystalline material which when recrystallised several times from pyridine yielded rhein melting at 320-321°. The sodium carbonate-insoluble material was well washed with acidulated water, crystallised many times from a mixture of ethyl acetate and chloroform (1 :1) and finally from pyridine when golden yellow chrysophanic acid melting at 193-194° was obtained.

Extract (B4).—Concentration gave yellow crystals with brownish granules. After repeated washing with ethyl alcohol the granules were

separated partly by hand-picking and partly by flotation. The yellow material on several crystallisations from dilute alcohol with norit carbon, became colourless, and was identified as rhaponticin (m.p. 230-231°). The brown granules when crystallised three times from alcohol melted at 207-208°, and on hydrolysis with dilute sulphuric acid gave a reducing sugar with impure chrysophanic acid. The alcoholic washings of the solid material described above gave emodin, m.p. 253°.

Summary.

The alcoholic extract of the rhizomes of *Rheum emodi,* Wall. has been found to contain the following :—Starch, tannin material, glucose, a small amount of volatile organic acids, an essential oil, chrysophanic acid, rhein, emodin, another hydroxyanthraquinone (probably aloe-emodin), the glucoside rhaponticin, and a mixture of glucosides of hydroxyanthraquinones from which chrysophanic acid glucoside has been isolated in a fairly pure form ; also oleic and linoleic acids, probably a mixture of palmitic and stearic acids along with a small amount of an unidentified acid of high molecular weight, phytosterol, brown resinous matter, and a large amount of brown amorphous powder resembling plant pigments.

Of the anthraquinone derivatives, chrysophanic acid was found to occur in the largest amount followed by emodin, rhein and the unidentified hydroxyanthraquinone derivative. The glucosides of hydroxyanthraquinones were also present in fairly large quantities. The minimum yield of rhaponticin was found to be about 0.7 per cent.

The results show that the Indian or Himalayan rhubarb contains most of the anthraquinone derivatives found so far in the official rhubarb (*Rheum palmatum,* Linn. or *Rheum officinale,* Baill.), together with an appreciable amount of the glucoside rhaponticin, which is absent from all the official rhubarb samples so far analysed. The Central Indigenous Drugs Committee (*loc. cit.*) found the drug unsatisfactory as a purgative but adduced no reasons for this. It would appear from the results of the present investigation that the griping action of the preparations from this drug is probably due to the presence of rhaponticin which is present to the extent of nearly one per cent. In order to throw more light on this aspect of the subject, rhaponticin is being subjected to a detailed pharmacological study, the results of which will be communicated in due course.

We desire to record our appreciation of the keen interest shown by Professor V. Subrahmanyan in the present investigation ; one of

us (M.G.M.) wishes also to express his grateful thanks to the Osmania University Council and the Principal of the Osmania Medical College, Hyderabad (Deccan) for having deputed him to work in these laboratories.

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DIAGRAMMATIC ANALYSIS OF A
INDIAN OR HIMALAYAN RHUBA

Steam-distilled

Volatile with steam extracted with ethyl ether

NaOH extract, chrysophanic acid

Ethereal residue, essential oil

Water-soluble (A) extracted with various solvents

Ethyl ether extract (A_1) residue dissolved in amyl alcohol and filtered

Residue, rhein

Filtrate extracted with alkalis

Na_2CO_3 extract, emodin

NaOH extract acidified; precipitate dissolved in chloroform and filtered

Filtrate extracted with alkalis

Residue, aloe-emodin (?)

0·5% NaOH, aloe-emodin (?)

5·0% NaOH, chrysophanic acid

Ethyl acetate extract (A_2) residue washed with alcohol and filtered

Residue, rhaponticin

Filtrate, glucoside of chrysophanic acid

Amyl alcohol extract (A_3) residue washed with alcohol and filtered

Residue, rhaponticin

Filtrate, glucoside of chrysophanic acid

Aqueous residue (A_4) treated with neutral and basic lead acetate solutions and filtered

Precipitate decomposed and extracted with solvents

Ethyl ether, organic acids

Amyl alcohol, mixed anthraquinone-glucosides

Filtrate, glucose

Petroleum ether (B_1) concentrated and filtered

Residue, chrysophanic acid

Filtrate concentrated, dissolved in ether and extracted with alkalis

Na_2CO_3 extract, indefinite

NaOH extract, chrysophanic acid

Ether extract crystallised from alcohol

Crystals, phytosterol

Residue, wax

Ethyl ether extra
concentrated
filtered

Filtrate extracted with alkali

Na_2CO_3 extr
emodin

Ether resi
saponified
and extrac
with ether

Soap
ac

Saturat
palmi
and ste