

## SHORT COMMUNICATIONS

BITTER PRINCIPLE OF SAXIFRAGA  
CILIATA

SALIMUZZAMAN SIDDIQUI AND M. MANZUR-I-KHUDA

*Central Laboratories, Pakistan Council of Scientific  
and Industrial Research, Karachi*

(Received February 20, 1961)

The plant *Zakhme Hayat* (*Saxifraga Ciliata*) is native to the northern region of Pakistan. It grows in Kashmir and is locally known as *Zakhme Hayat*. The roots of this plant are used locally as an astringent, tonic and stomachic.

No chemical investigation on this plant has been reported so far in the literature. However a number of species belonging to *Saxifragaceae* has been investigated, which includes *Astilbe Thunbergi* Miquel, *A. formosa* Nakai, *A. odontophylla* Miq. var. *Congesta* Hara, *Rodgersia Podophylla* A. Gray, *Peltoboykinia tellinoides* Hara, *Bergenia stachevi* Engler<sup>1</sup> and *Saxifraga Crassifolia*.<sup>2</sup>

A polyphenollic compound  $C_{14}H_{16}O_9 \cdot H_2O$  designated as 'bergenin' has been reported from the above species.

Some Indian workers have reported some work on *Zakmihayat*<sup>3</sup> from Kashmir. But this plant (*Cissampelos Pareira* Lim) though has the same local name, is different from the one we have been working on.

The present investigation of the plant was aimed at isolating and identifying the active principle of *Saxifraga ciliata* collected from the northern districts of West Pakistan.

The fresh roots were cleaned and chopped into  $\frac{1}{2}$  inch pieces and dialysed with 95% ethanol followed by 80% and finally 70% ethanol in water. The dialysate was dried in vacuo to give a dark red glassy mass. This was dissolved in water and extracted with ethyl acetate. The aqueous layer deposited prismatic crystals on standing. Further crops of crystals could be obtained by gradual concentration of the aqueous layer. It was found that if the concentration was increased quickly only a viscous precipitate could be obtained on cooling. The ethyl acetate layer on

concentrating also gave some crystals which were identical with those obtained above. The total yield of these crystals from the roots were 0.85% (moisture free basis). This material is very similar to bergenin (analytical data), some physical constants and reaction characteristic as can be seen from the following tabulated data are also similar. However, there is a large difference in optical rotation value of the sample isolated by us and that reported in the literature.

	Bitter from <i>Saxifraga Ciliata</i>	Bergenin Lit. value
m. p.	152-3°C. and 244°C.	148-9° and 234°C
$[\alpha]_D^{25}$	-70° at 22°C. (1% in abs. EtOH)	-37.25° (0.99% EtOH Sol.)
Crystalline form	Cubes from water	—
Solubility	Soluble in hot water, EtOH, MeOH and EtOAc. Insoluble in all other usual solvents.	Same as the other
Bitterness	Detectable upon a dilution of 1 in 10,000 (water).	—
Mol. formula	$C_{14}H_{16}O_9 \cdot H_2O$	$C_{14}H_{16}O_9 \cdot H_2O$
Functional groups	6 -OH and I-MeO. No COOH.	6 -OH and I-MeO No COOH.
Derivatives	Penta-acetate m. p. 209-10° $[\alpha]_D^{25} -96^\circ$	Penta-acetate m.p. 199-203°C

## Experimental

*Dialysis of the Roots.*—The fresh cleaned roots were chopped into  $\frac{1}{2}$  inch pieces (2 kg.; 56% moisture) and soaked with 99% ethanol for 3 days. This process was repeated once more with 95% ethanol and then with 80% ethanol followed by 70% ethanol. The total extracts were concentrated in vacuo and finally dried to yield a dark red highly viscous mass (168 g.).

*Working up of the Dried Dialysate.*—The dried extract (149 g.) was distributed between ethyl acetate (250 ml.) and water (300 ml.). The aqueous layer was extracted twice (125 ml.  $\times$  2) with ethyl acetate and then cooled in refrigerator (I). It deposited a dark brown mass which on dissolving in water (300 ml.) and cooling yielded a crys-



talline material (1.0 g., m.p. 150-65°C.). This on recrystallising from the same solvent (charcoaled) gave crystals m.p. 152-3°C. and 244°C.

The aqueous mother liquor (from I) on concentrating slowly in four stages yielded crystals at every stage totalling 7.25 g. (1.15 g., 3.5 g., 0.1 g., and 2.5 g., m.p. 150°C.). On recrystallising from the same solvent (charcoaled) it gave crystals m.p. 152-3°C. and 244°C., (colourless cubes)  $[\alpha]_D^{25} - 7.0^\circ$  (1% Soln. in absolute ethanol).

The ethyl acetate (500 ml.) extract on concentrating to 100 ml. volume also gave crystals of the above compound (0.25 g., m.p. 150°C.).

The bitterness of the above compound was determined by gradual dilution with water until no more bitter taste could be obtained and was found to be detectable upto a dilution of 1 in 10,000.

The purified bitter, m.p. 152-3°C. and 244°C., analysed as  $C_{14}H_{16}O_9 \cdot H_2O$ . (Found C, 48.25, 48.29; H, 5.25, 5.49; O, 46.05; Act.  $H^+$ , 2.15; OMe, 8.85; C- $CH_3$ , 0.00%;  $CH_3CO$ , 1.1%. Mol. wt., 333.  $C_{14}H_{16}O_9 \cdot H_2O$  requires C, 48.55; H, 5.25; O, 46.2; Act.  $H^+$ , 2.2 (for 7 act.  $H^+$ ); OMe, 8.95; (for one OMe); C- $CH_3$ , 0.00 (No C- $CH_3$ );  $CH_3CO$ , 12.4% (for one  $CH_3CO$ , 0.0 for none). Mol. wt., 346.25).

*Acetylation of the Bitter.*—The bitter (150 g.) was dissolved in acetic anhydride (3 ml.) and pyridine (3 ml.). It was heated at 100°C. for one hour and then evaporated to dryness in vacuo (250 g.). It crystallised from benzene petroleum ether (60-80°) mixture as leaflets, m.p. 209°C. Once more crystallised (charcoaled) it gave m.p. 209-10°C. The acetylated bitter analysed as  $C_{24}H_{26}O_{14}$ . (Found C, 53.75, 53.95; H, 5.25, 5.0; O, 40.6;  $CH_3CO$ , 40.9%  $C_{24}H_{26}O_{14}$  requires C, 53.35; H, 4.9; O, 41.6;  $CH_3CO$ , 39.8 (for 5- $CH_3CO$ )%  $[\alpha]_D^{25} - 9.6^\circ$  (1% solution in  $CHCl_3$ ).

### Summary

The constituents of plant *Zakhme Hayat* (*Saxifraga ciliata*) has been investigated and a bitter principle closely resembling bergenin isolated from it.

The crystalline bitter (m.p. 152-3°C. and 244°C.) is a polyphenolic compound ( $C_{14}H_{16}O_9 \cdot H_2O$ ) with 6-OH and 1-MeO and a lactone group.

### References

1. T. Inove and T. Yamaki, *Misc. Repts.*

*Research Inst. Nat. Resources* (Japan), **10**, 13-17 (1946).

2. I. Bergenin, A. E. Chichibabin, A. V. Kirsanov, A. I. Korelev and N. N. Vorozhtov Jr., *Ann.*, **469**, 93-127 (1929).
3. S. Bhattacharji, V. N. Sharma, M. L. Dhar, *J. Sci. Ind. Research* (India), p. 81 (1952).

## STUDIES ON TROMBIDIUM TINCTORUM LINN.

### Part II.—Chemical Constitution of the Non-Saponifiable Matter from the Fat of *Trombidium Tinctorum* Linn.

SAHFIQ AHMAD KHAN, MUHAMMAD KHURSHID BHATTY AND KARIMULLAH

*Oils, Fats & Waxes Research Division, West Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Lahore*

(Received April 5, 1961)

In an earlier paper,<sup>1</sup> it has been shown that the saponifiable portion of the petroleum ether extract of *Trombidium tinctorum* Linn. (mites) contained butyric, iso-valeric, caproic, lauric, myristic, palmitic, stearic and  $C_{14}$ ,  $C_{16}$ ,  $C_{18}$  and  $C_{20-22}$  unsaturated acids. The present communication deals with the non-saponifiable matter.

The non-saponifiable material was isolated by extraction with diethyl ether in a suitable extractor. The red coloured non-saponifiable matter so extracted was subjected to crystallisation which furnished different crude fractions consisting of two colourless crystalline substances and one oily pigment

The two colourless substances were purified through repeated crystallisation and then by alumina column chromatography. The pigment was purified by crystallisation only because it showed strong adsorption on alumina. In this manner three distinct substances were obtained. The first was amorphous and had waxy appearance; the second was a crystalline substance having white colour while the third was a crystalline pigment which had an intense red colour.

### Experimental

The non-saponifiable matter, 2.6% on the basis of the weight of the oil, was dissolved in



methanol, when a yellowish white mass was obtained by keeping the solution overnight at room temperature. The mass was filtered out as Fraction 1 and the filtrate which was coloured bright red was concentrated under reduced pressure, when a second fraction of shining white plates was obtained. These plates were filtered and washed with methanol to remove the adherent colouring matter. The filtrate (residue) was taken as fraction 3 as it did not yield any further crystalline material.

**Fraction 1.**—The waxy mass (1.03%) was dissolved in hot ethanol (95%). On cooling, a white amorphous substance (m.p. 69.5°C.) was isolated. It was repeatedly crystallised from ethyl acetate. No change in its melting point was however, noticed. This substance did not respond to reactions characteristic of sterols such as Liebermann-Burchard and Salkowski tests. The substance was found by various tests to be a higher saturated alcohol.

Its benzoyl derivative was obtained in shining flakes, m.p. 40°C. The acetyl derivative was also a wax-like substance, m.p. 37-38°C. The phenyl urethane of this alcohol was also prepared. Its m.p. was observed to be 87°C. From the derivatives, this fraction was identified as docosyl alcohol.<sup>2</sup>

**Fraction 2.**—The second fraction (1.3%) was a sterol as it responded positively towards Liebermann-Burchard and Salkowski tests. It was purified by adsorption over alumina and elution with benzene - chloroform (3:2 v/v). The sterol had a sharp melting point, 146°C.,  $[\alpha]_D - 39^\circ$  ( $\text{CHCl}_3$ ). The following derivatives of the sterol were also prepared and studied.

Acetyl, m.p. 112-113°C.,  $[\alpha]_D - 47^\circ$  ( $\text{CHCl}_3$ ).  
Benzoyl, m.p. 141-142°C.,  $[\alpha]_D - 13.5^\circ$  ( $\text{CHCl}_3$ ).  
Bromide, m.p. 115°C.,  $[\alpha]_D - 43^\circ$  ( $\text{CHCl}_3$ ).

The sterol digitonide was obtained as a white powder, m.p. 234°C., (decomp.). The sterol on regeneration from the complex had all its original characteristics. It was thus identified as cholesterol.<sup>3</sup>

**Fraction 3.**—The coloured filtrate, residue of fractions 1 and 2, was freed from the solvent under reduced pressure. It (0.27%) responded positively towards tests characteristic of carotenoids, e.g., blue colour with concentrated sulphuric acid, blue-green colouration with antimony trichloride in chloroform, etc. The residue was crystallised separately from methanol and pyridine and water. The needles melted at 240°C., while their acetyl derivative at 233°C. The pigment gave a characteristic absorption spectrum at 500-505 m $\mu$  when

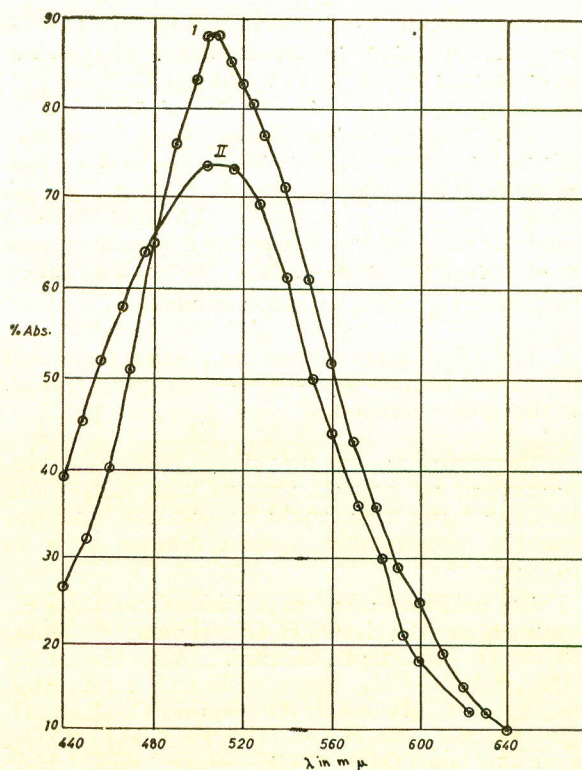


Fig. 1

studied spectrophotometrically in carbon disulphide (Fig. 1). From these studies it was concluded that the pigment is astacene.<sup>5</sup> The pigment can also be partially separated from the ether/water interface at the time of extraction of the non-saponifiable matter. It can be regenerated from its alkali salt as mentioned in the literature.<sup>4</sup>

### Summary

The non-saponifiable matter has been shown to contain docosyl alcohol, cholesterol and astacene. The substances have been characterized by studying their physical as well as chemical behaviour.

**Acknowledgements.**—Our thanks are due to Mr. Iftikhar Ahmad of West Regional Laboratories for his help and to Dr. Salimuzzaman Siddiqui, F. R. S., for his keen interest in this problem.

### References

1. S. A. Khan, M. K. Bhatti, and Karimullah, Pakistan J. Sci. Ind. Research, **3**, 6 (1960).
2. I. Heilbron, and H. M. Bunbury, *Dictionary of Organic Compounds*, Vol. II.



- (Eyre and Spottish woode London, 1953) p. 455.
3. R. T. Holman, W. O. Lundberg and T. Malkin, Progr. in Chem. Fats Lipids. **1**, 41 (1952).
  4. K. Paech and M. V. Tracey, *Modern Methods of Plant Analysis* Vol. III. Springer — Verlag Berlin Gottingen Heidelberg. (1955) p. 382.
  5. P. Karrer, and E. Jacker, *Carotenoids*, Elsevier Publishing Company, Inc., New York, p. 237 (1950).

### UTILIZATION OF COTTONSEED FATTY ACID PITCH FOR THE PREPARATION OF BLACK STOVING ENAMEL

MOHAMAD ASLAM AND G. MUSTAFA ALI

*Paints and Plastics Research Division, Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi.*

(Received February 28, 1961)

The residue remaining after the removal of volatile fatty acids (prepared from soap stock obtained during refining of oil by caustic soda) is termed pitch and is composed of non-volatile fatty acids, polymerised fatty materials, unsaponifiable matter originally present in the feedstock and other impurities not completely identified. The chief use of this pitch is reported to be in the preparation of varnishes, lacquers and japans, for which purpose it may be used as such or in admixture with drying or semi-drying oils, bitumin, coal-tar, and coal-tar pitch. The present communication gives the results of a study undertaken to utilize the fatty acid pitch for the preparation of a black stoving enamel comparable in quality with an imported product (stoving cycle enamel, black finishing). Fatty acid pitch used in this investigation was supplied by Sadiq Ali Vegetable Oil and Allied Industries, Bahawalpur.

The cottonseed fatty acid pitch used in the present investigation has two important characteristics: (1) when heated above temperature of 120°C., it gels and becomes insoluble in solvents; (2) it dissolves in solvents at 80-90°C., leaving 8-10% of insoluble matter. The normal method of mixing the ingredients by fusion for the preparation of varnish could not be used. The pitch therefore was dissolved in mineral turpentine, centrifuged to remove insoluble matter and the solution adjusted to contain 60% solid matter.

The formulations which have been investigated are presented in Table 1.

#### Method of Preparation

For formulation Nos. 1 to 3, 60% solutions of pitch and ester-gum are mixed in a ball mill for about four hours. In case of formulation No. 4, carbon black is first dispersed into a small quantity

TABLE 1.—FORMULATIONS OF STOVING ENAMELS  
DRIER: 1.0% Pb, 0.1% Mn BY WEIGHT OF  
PITCH.

Formulation No.	Fatty acid pitch parts by weight	Ester-gum parts by weight	Carbon black % by weight of pitch
1	100	—	—
2	100	25	—
3	100	50	—
4	100	25	2

TABLE 2.—SYNOPSIS OF TESTS ON THE  
FORMULATIONS OF TABLE 1.

Formulation No.	Baking temperature °C.	Scratch hardness	Gloss	Colour
1	125	400	76	brownish black
2	125	700	84	dark brown
3	125	700	84	dark amber
4	125	650	88	jet black
Imported	125	500	65	jet black
4	150	500	70	jet black
Imported	150	650	74	jet black
4	175	500	65	jet black
Imported	175	700	80	jet black



of the pitch on a triple roller mill to secure a uniform paste which is finally mixed with other ingredients in a ball mill.

For testing, the enamels were applied on tin panels and then baked in ovens at 125°C., 150°C., and 175°C., for two hours. Scratch hardness of the baked films has been determined as the load in grams required to rupture the film and the gloss has been measured on the glossmeter. The results are reported in Table 2, together with comparative

data for the imported product.

### Conclusion

From the results, it is obvious that formulation No. 4, i.e. fatty acid pitch containing ester gum 25%, Pb 1.0%, Mn 0.1%, baked at 125°C. for two hours is comparable in performance with the imported cycle stoving enamel baked at 175°C. for 2 hours.