MUSTARD OILS AND MUSTARD OIL GLUCOSIDES OCCURRING IN THE SEED KERNELS OF *PUTRANJIVA ROXBURGHII* WALL.

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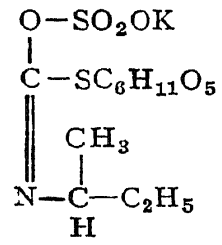
Gambhir and Dutt[1] have reported the presence of phenyl mustard oil (phenyl-*iso*-thiocyanate) in the fatty oil from the seeds of *Putranjiva roxburghii* Wall. (Family Euphorbiaceæ) which the earlier work of Krishna and Puntambekar,[2] had not recorded. To find the possible cause of this discrepancy, therefore, the oil, obtained both by petroleum-ether extraction and by cold hydraulic expression of the putranjiva kernels at different stages of their development, was re-examined. It was discovered that the "mustard" smell was highest in the fatty oil (mustard oil content being 35% of the amount present in the kernels) from the kernels of fully grown but green fruit, medium in those of the ripe fruit, and negligible in those that had fallen on the ground for a long period.[3] It was further observed that in every case when the kernels were extracted with petroleum-ether, they yielded four times as much "mustard" constituent in the fatty oil as that obtained by cold hydraulic expression. From the findings recorded in Table I it is clear that the occurrence of mustard oil along with the fatty oil depends upon the moisture in the kernels. If the kernels are ripe but wet, the mustard oil which is liberated by enzymic hydrolysis of the component glucosides is produced in large quantity, but if the kernels are dry the mustard oil is absent. If, therefore, dry kernels are used, as they usually are, for the extraction of the fatty oil, the mustard oil is missing. This explains the reason for our having missed the mustard oil in our earlier work.[2]

During the course of the present investigation the oil which has been designated as mustard oil turned out to be a mixture of phenyl, *iso*propyl and *sec*-butyl mustard oils. Of these *iso*propyl mustard oil forms the largest part (approx. 70%) and phenyl mustard oil the least (approx. 3%). These are separated through fractional distillation and identified through their thiourea derivatives.

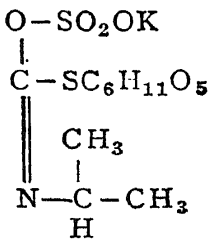
The pulp of the fruit and the leaves were also examined for the possible presence of mustard oils or their glucosides, but none were found. The pulp, however, yielded a large proportion of mannitol and a small amount of amorphous non-sulphur glucoside from which a crystalline aglucon has been isolated and is under further examination.

Out of the three mustard oils found in putranjiva seed kernels, only secondary butyl mustard oil has been recorded in literature[4] to occur in the form of its glucoside, gluco-cochlearin, in the leaves, seeds and flowers of *Cochlearia officinalis* Linn. and in the leaves, stems and roots of *Cardamine amara* Linn. and *C. pratensis* Linn. The glucoside, however, has not been isolated in pure form. The presence of the other two mustard oils, namely, *iso*propyl and phenyl mustard oils, has not so far been reported in any other plant.

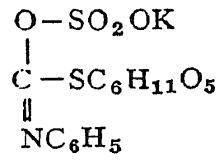
Of the few mustard oil glucosides reported in literature only sinigrin and sinalbin from the seeds of *Brassica* sp. and glucocheirolin from the seeds of *Cheiranthus* sp., all belonging to the Cruciferæ family, have so far been isolated in pure form. The others owing to their hygroscopic nature and to the difficulty involved in their separation from accompanying sugars and glucosides, have been identified through their products of hydrolysis and characteristic reaction products with silver nitrate. To this category belong the following glucosides, namely, gluconasturtiin from the seeds of *Nasturtium officinale* R.Br.; glucotropæolin from the seeds of *Tropæolum majus* Linn. and *Lepidum sativum* Linn. and the abovementioned gluco-cochlearin from the seeds, leaves and flowers of *Colchlearia officinalis* Linn. Purification of these glucosides by means of lead acetate and subsequent deleading with hydrogen sulphide has been found to bring about their decomposition. In the present work also the same difficulty of isolation of the three mustard oil glucosides in pure form was experienced. Thus their fractional crystallization from alcohol and alcohol-ether mixture was not successful, nor was it found possible to free them completely from accompanying reducing compounds. Their presence could be proved directly only by the study of the general properties of the mustard glucosides, namely, enzymic and acid hydrolysis and formation of silver salt, and indirectly by the isolation of individual mustard oils, as described above. The finding of dextrose and potassium acid sulphate among the products of acid hydrolysis of the mixed glucosides completes their characterization and suggests their formulæ. Out of the three glucosides the *sec*-butyl mustard oil glucoside is already known as glucocochlearin[4] and the names for *iso*propyl and phenyl mustard glucosides are now proposed as follows:—

(1) 

Sec.-butyl mustard oil glucoside or glucococchlearin

(2) 

*Iso*propyl mustard oil glucoside or glucoputranjivin

(3) 

Phenyl mustard oil glucoside or glucojiaputin

It is well known that the medicinal properties of the mustard seed (mustard plaster) are due to the action of the mustard oil (allyl *iso*thiocyanate) liberated in the presence of moisture from the glucoside sinigrin contained in them. This mustard oil is stated to be a powerful rubefacient. Diluted either with alcohol or lard it is applied as a counter-irritant to sprains and is used for rheumatism and similar conditions. Morel[5] found that allyl *iso*thiocyanate has marked bacteriostatic powers especially against the tubercle bacillus. The volatile mustard oil has been used as a substitute for mustard plaster; for this purpose one part of it may be mixed with 60 parts alcohol and the mixture sprinkled not too thickly on muslin and covered with waxed paper. It is said to act quickly and effectively. In view of the fact that the stones of the putranjiva fruit in the form of rosaries are hung round the necks of young children to keep them in good health, it may be that the protection against the diseases of childhood is actually given by one or all of these three mustard oils slowly liberated from the glucosides of the seeds in contact with body moisture.

Mustard oils and mustard oil glucosides have been commonly found in plants of the family Cruciferæ, particularly its genus *Brassica*. They have also been reported to occur, although sparingly, in plants of the families Capparidaceæ, Tropæolaceæ, Salvadoraceæ and Resedaceæ. No record, however, exists of their presence in the family Euphorbiaceæ to which *Putranjiva roxburghii* Wall. belongs.

Experimental

(*a*) *Examination of the Fatty Oil for Mustard Oils*

Putranjiva fruits were collected locally in the following manner:— (1) Fully grown, unripe and plucked from trees, (2) ripe and plucked from the trees, and (3) fallen on the ground. After removal of the pulp the stones were crushed and the kernels handpicked. It was noticed that the moist kernels from the fully grown but unripe fruit gave a strong mustard smell, those of the ripe ones gave a mild mustard smell but those from the

fallen fruit (air-dried) gave no smell at all. When, however, the kernels of the fallen fruits were moistened and left for some time the mustard smell developed. In order to prove this in a quantitative way each group (600 g.) of kernels was divided into two equal parts and the fatty oil from the one was obtained by cold expression and from the other by petroleum ether (60-80° C.) extraction and sulphur content (which is really the index of mustard oil present) of each determined by Carius method. The data are recorded in the table below:—

TABLE I

Sample	Condition of fruit	Method of extraction of fatty oil from kernel	Sulphur content of the fatty oil (Carius)
1 (*a*)	Fully grown unripe and plucked from trees	Petroleum-ether (60–80°)	1·05%
1 (*b*)	do ..	Cold hydraulic expression	0·26%
2 (*a*)	Ripe and plucked from trees ..	Petroleum-ether (60 80°)	0·55%
2 (*b*)	do ..	Cold hydraulic expression	0·14%
3 (*a*)	Fallen on ground and air-dried ..	Petroleum-ether (60–80°)	0·05%
3 (*b*)	do ..	Cold hydraulic expression	Traces

The sulphur content of the kernels of the air-dry seeds fallen on the ground was found to be 1·7% (on dry basis). This corresponds to the presence of 10·8% of the mixed mustard glucosides or 2·8% of the mixed mustard oils in the kernels with sulphur content of 15·7% and 26·32% respectively. It was therefore expected that on complete hydrolysis of the mustard glucosides in 100 g. of the dry kernels 2·8 g. of the mixed mustard oils would be liberated and subsequently extracted along with the fatty oil of the kernels by the organic solvent used for the purpose. Theoretically therefore the fatty oil (25% in the dry kernel) thus extracted should have four times the sulphur content, namely, 2·95% of the total quantity of mustard oils completely liberated from 100 g. of dry kernel. Actually however under the comparable natural conditions as recorded in Table I 3 (*a*), for the seeds fallen on ground and air dried the sulphur content of the solvent extracted fatty oil from the kernels has been found to be 0·05% indicating thereby that only 1·7% of the mustard oils present in the kernels is extractable along with the fatty oil by an organic solvent. Compared to these figures the corresponding figures for the sulphur and mustard oil content of the kernels from fully grown unripe fruit plucked

from trees [Table I 1(*a*)] turn out to be 1·05% and 35% respectively. It might be pointed out in this connection that the yield of mustard oil in the kernels by the fermentation method described below is 0·5% and which amounts to 18% of the total mustard oil actually present in them.

(*b*) *Isolation of Mustard Oils from the Wetted Kernels*

In order to obtain the maximum quantity of mustard oils the following procedure was adopted:—

300 g. of the kernels were reduced to a fine paste, with water, and allowed to stand, in a closed vessel, overnight. Strong mustard smell developed. The mass was then steam-distilled, the distillate saturated with common-salt and extracted with sulphuric ether. The ethereal extract was dried over anhydrous sodium sulphate, ether removed and 1·5 g. of a sharp-smelling brownish-yellow mustard oil (yield 0·5%) obtained. Working in lots of 300 g. a total of 40 g. of the mustard oil of the following characteristics was collected from 8 kilos of kernels:—

Specific gravity at 25° C.	1·027
Refractive index at 25° C.	1·5187
Specific rotation $[\alpha]_D^{25°C.}$ (C = 5% in 95% alcohol)	+ 15°

(*c*) *Fractional Distillation of the Mustard Oil*

36 g. of the oil was fractionally distilled in a 50 c.c. Claisen flask at atmospheric pressure and the following fractions were collected:—

TABLE II

Fraction	B.P.(°C.)	Smell	Weight	Sp. G. at 20° C.	$[\alpha]_D^{25°C.}$	Remarks
0	Below 60	..	1·2 g.	..	..	Mostly ether sulphuric
1	135—45	Strong mustard	18 g.	0·9365	+10·9	Boils mostly at 136–38° C.
2	145—55	do	10 g.	0·9697	+26·0	Boils mostly at 153–55° C.
3	155—65	Mustared+Repulsive	1·8 g.	0·9900	..	Slight decomposition
4	Residue	Repulsive	5 g.	1·5102	..	Decomposed material

Fraction 0 had a mixed smell of mustard and sulphuric ether. It consisted mostly of the residual sulphuric ether with which the mustard oils were extracted from the steam distillate and a little mustard oil also distilled along with it. The mixture was treated with 10% ammonia and slowly

evaporated. Crystals of a thiourea were obtained and identified by its melting point and unaltered mixed melting point with *iso*propyl thiourea prepared from fraction 1.

Fraction 1 was refractionated and the major portion boiling at 136–38° C. collected separately. The thiourea derivative of this had the melting point 164–65° C. (found: S, 27·4%; *iso*propyl thiourea $C_4H_{10}N_2S$ requires S, 27·1%). The presence of *iso*propyl mustard oil was confirmed as follows:

A small portion of the refractionated material was hydrolysed by refluxing for three hours with 20% HCl, whereby *iso*propylamine hydrochloride, carbon dioxide and hydrogen sulphide (lead acetate test) were obtained.

$$(CH_3)_2\,CH - N = C = S + 2H_2O + HCl \rightarrow (CH_3)_2\,CH - \\ - NH_2\,HCl + CO_2 + H_2S$$

The hydrolysate was treated with chloroplatinic acid and evaporated to a small volume when beautiful shining small orange flakes of m.p. 228–30° C. crystallised out. Their mixed melting point with pure synthetic *iso*propyl amine chloroplatinate remained unaltered. The optical rotation of the fraction was due to the presence of a small amount of another mustard oil (secondary butyl mustard oil) identified in fraction 2. The fraction is thus mostly *iso*propyl mustard oil with a small quantity of *sec*-butyl mustard oil.

Fraction 2 was refractionated and the major portion boiling at 157–60° C. was collected. The thiourea derivative of this had the m.p. 137–38° C. (found: S, 24° 4%; $C_5H_{12}N_2S$ requires S, 24·2%). The presence of secondary butyl mustard oil was confirmed as follows:—

A small part of the refractionated material was hydrolyzed by refluxing for three hours with 20% HCl, yielding *sec*-butyl amine hydrochloride, carbon dioxide and hydrogen sulphide.

$$CH_3 - CH_2 - \underset{\displaystyle CH_3}{\underset{|}{CH}} - N = C = S + 2H_2O + HCl \rightarrow CH_3 - CH_2 - \underset{\displaystyle CH_3}{\underset{|}{CH}} - \\ - NH_2 \cdot HCl + CO_2 + H_2S$$

The hydrolysate was treated with chloroplatinic acid, and was evaporated to a small volume when golden yellow plates crystallised out, m.p. 208–10° C. Their mixed melting point with pure synthetic *sec*-butyl amine chloroplatinate remained unaltered. The correct sulphur content but lower specific rotation of the thiourea derivative [found $(\alpha)_D^{25°\,C.} + 10$, requires

$[\alpha]_D^{25° C.} + 22·77]$ of the fraction indicates the presence of racemic *sec*-butyl thiourea and consequently of racemic *sec*-butyl mustard oil.

Fraction 3 had a milder smell of mustard as well as a slightly unpleasant smell of sulphur compounds, probably due to some pyrolysis of the mustard oil. On converting a small portion to thiourea, crystals of indefinite melting point, 130–50° C. were obtained. The fraction was, therefore, distilled a couple of times with alcohol to remove any volatile constituents. Both the distillate and the residue were treated with 10% ammonia and evaporated to dryness. The thiourea so formed on crystallisation from water melted at 139–40° C. and 153–54° C. respectively. The melting point of the former and its unchanged mixed melting point with thiourea isolated from fraction 2 established its identity with *sec*-butyl thiourea prepared from fraction 2. The melting point of the latter, its unchanged mixed melting point with phenyl thiourea from synthetic phenyl mustard oil and its sulphur content (found: S, 21·5%; $C_7H_8N_2S$ requires S, 21·0%) established its identity as phenyl thiourea. The fraction is thus a mixture of *sec*-butyl and phenyl mustard oils.

Residue was a thick dark repulsive smelling oil containing sulphur and appeared to be some pyrolytic products of mustard oils. It did not yield any thiourea.

(*d*) *Isolation of the Mustard Oil Glucosides from the Kernels*

300 g. of kernel powder of the air-dried seeds fallen on the ground was first extracted with petroleum ether in a soxhlet to remove the fatty oil, then thrice successively with boiling 90–95% alcohol and the alcohol removed under vacuum. The viscous residue was taken up in cold water and filtered from the waxy precipitate. The filtrate was shaken with a little barium carbonate to remove any free acidic substances, heated to boiling, cooled, filtered again, decolourized with animal charcoal, reduced to a small volume under vacuum and allowed to crystallise. Since crystallisation did not take place in cold from single or mixed solvents its purification was tried by dissolving it thrice successively in 95% alcohol and precipitating it out by addition of ether. Even at this stage the material showed the presence of reducing compounds with Fehling's solution. The product was dried in vacuum at 50° for 4 hours and further left in vacuum for several days. It was a pale-brown, amorphous highly hygroscopic bitter substance and gave a strong test for sulphur. It was soluble in alcohol and water but insoluble in ether, chloroform and benzene. With neutral lead acetate it gave a slight precipitate. With basic lead acetate a thick white precipitate

was formed after addition of ammonia to the mixture. The water solution was dextrorotatory $[\alpha]_D^{25° C.} + 18 \cdot 51$ (C = 3·46%). With silver nitrate (10%) it gave a thick dark violet precipitate which dissolved in ammonia, a characteristic property shown by all the mustard glucosides so far isolated from natural sources (0·1500 g. of the silver salt gives 0·1080 g. AgCl, theory requires 0·1025 g. AgCl). On slight warming with 10% HCl it was hydrolysed and the characteristic strong smell of mustard oil was produced. Amongst the other products of hydrolysis potassium acid sulphate and dextrose were identified in the following manner:—

Potassium acid sulphate.—The dry kernel powder was first extracted with petroleum ether to remove the fatty oil. It was then wetted with water and left in an incubator overnight at 38°. After a couple of hours the characteristic smell of mustard developed and increased with the length of time. On the following day, *i.e.*, after 20 hours, the mixture was thoroughly extracted with ether to remove the mustard oil that was formed. After two further successive overnight incubation at 38° C. and ether extractions to remove any mustard oil, the residual kernel paste was diluted with water, the mixture filtered and the residue on the filter was further washed a couple of times with water. The total filtrate on decolourisation with animal charcoal and concentration nearly to dryness under vacuum deposited a large amount of heavy gritty white crystals. These were extracted with warm alcohol (95%) twice to remove all the reducing sugars and the crystalline gritty residue recrystallised from water. On analysis it turned out to be potassium acid sulphate.

Dextrose.—The alcoholic extract was reduced to a small volume under vacuum at 70° C. A portion of the residue was taken up in water decolourised and made to 100 c.c. in a volumetric flask. Its specific rotation was found to be $[\alpha]_D^{30° C.} + 48°$ (C = 3·1%). It reduces Fehling's solution (5 c.c. gave 0·3260 g. Cu_2O, calculated for glucose 0·3360 g. Cu_2O). The sugar was identified as dextrose by the formation of phenyl osazone (m.p. 207–08°) with phenyl hydrazine and of saccharid acid by oxidation with nitric acid.

Summary

Besides phenyl mustard oil reported by Gambhir and Dutt two other mustard oils, namely *iso*propyl and *sec*-butyl *iso*-thiocyanates, have been isolated from the steam volatile oil (yield 0·5%) of the wet kernels of *Putranjiva roxburghii* Wall. All the three mustard oils (69% isopropyl, 28% *sec*-butyl and 3% phenyl mustard oils) are derived from glucosides present in the seed kernels and are only liberated from them in the presence of moisture

by enzymic hydrolysis along with dextrose and potassium acid sulphate. Under these moist conditions it is found that as much as 35% of the mustard oils are extracted by petroleum-ether from the kernels along with the fatty oil but only to the extent of 1·7% if the kernels are dry. Fatty oil expressed from dry kernels contain only traces of the mustard oils.

Out of these mustard oils only *sec*-butyl mustard oil has been previously reported to occur in the plants of the Cruciferæ or mustard family but all the three are now found for the first time in a plant of the Euphorbiaceæ family. The characteristic mustard smell of the moist kernels is due to *iso*propyl and *sec*-butyl mustard oils, the principal constituents of the steam volatile oil isolated from them.

While mustard oil glucosides have been isolated in the form of their mixture their individual isolation has not been possible owing to their highly hygroscopic nature and due to the difficulty of obtaining them in the crystalline state.

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