

Isothiocyanates XLIII *. Isothiocyanates from *Putranjiva Roxburghii* Wall. Including (S)-2-Methylbutyl Isothiocyanate, A New Mustard Oil of Natural Derivation

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The isothiocyanates produced on enzymic hydrolysis of glycosidic progenitors in seed kernels of the Indian tree *Putranjiva Roxburghii* Wall. (*Euphorbiaceae*) have been reinvestigated. Previous findings of isopropyl and 2-butyl isothiocyanate have been confirmed whereas the volatile hydrolysis product of the minor glucoside, *glucojiaputin*, is demonstrated to be the previously unknown (S)-2-methylbutyl isothiocyanate (V), and *not*, as formerly claimed^{3,4}, phenyl isothiocyanate.

The structure of the new isothiocyanate is established by analysis, optical activity, and n.m.r. spectroscopy of the corresponding thiourea. Its absolute configuration appears from a stereoselective synthesis departing from (S)-2-methylbutanol.

In addition to the three volatile mustard oils, identified also by gas chromatography, an enzymically hydrolyzed seed extract is shown to contain (-)-5-ethyl-5-methyl-2-oxazolidinethione (cleomin) (VI), arising from the glucoside *glucocleomin* which recently was established as a wide-spread constituent in species of the family *Capparidaceae*¹³.

The isothiocyanate glucosides with branched side-chains encountered thus far are summarized and possible biogenetic pathways discussed.

The tree *Putranjiva Roxburghii* Wall. of the family *Euphorbiaceae* is indigenous to and cultivated as an ornamental throughout tropical India. Since antiquity, curative effects have been attributed to its leaves and seeds, administered in form of decoctions for oral use or as poultices for local application^{1,3}. Again, stones of the fruit are reputed to be protective against diseases when placed as rosaries around the necks of children^{1**}.

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** In fact, the vernacular names for the tree (*e.g.* Sanskrit *Putra-jiva*, Hindi *Putranjiva* or *Jiputa*) all signify "the life of the child".

Although the high oil content of *Putranjiva* seeds was recognized long ago¹, it was not until 1931 that Krishna and Puntambekar² made the first study of the composition of the glycerides. Gambhir and Dutt³ later extended this investigation and reported the presence in the seed oil of 0.7 % of phenyl isothiocyanate, to which the therapeutic properties of the otherwise normally composed vegetable oil were attributed. The Indian authors based their identification of the steam-volatile mustard oil solely on a sulphur analysis of the corresponding thiourea derivative, m.p. 52°, seemingly inadvertent of the discrepancy between this value and the m.p. 154° of authentic phenylthiourea. This statement prompted Puntambekar⁴ to undertake a more detailed study of the steam-volatile isothiocyanates produced on enzymic hydrolysis of glycoside progenitors in seeds of *P. Roxburghii*. Upon fractional distillation, three individual components were partly separated, viz. isopropyl isothiocyanate, partly racemized (+)-2-butyl isothiocyanate, and small quantities of a higher-boiling fraction which was claimed to be phenyl isothiocyanate on basis of the sulphur content of the corresponding thiourea. This was reported to melt at 153–154°, alone or in admixture with authentic 1-phenylthiourea. Whereas (+)-2-butyl isothiocyanate, the enzymic hydrolysis product of *glucocochlearin*, occurring in, e.g., several species of the family *Cruciferae* (cf. Ref.⁵), presented no novelty within the plant kingdom, the other two isothiocyanates had not formerly been recognized as plant constituents. Puntambekar⁴ introduced the designations *glucoputranjivin* and *glucojiaputin* for the glucosidic progenitors affording isopropyl and phenyl isothiocyanate, respectively. The former has since been repeatedly encountered in this laboratory, notably in species of the family *Cruciferae*⁵, whereas no other well-authenticated source of phenyl mustard oil has since been reported*.

In connexion with current studies on the distribution and structure of isothiocyanates of natural origin it became of interest to reinvestigate *Putranjiva Roxburghii* Wall., particularly because this plant represents the sole well-documented source of isothiocyanate-producing glycosides within *Euphorbiaceae***.

This family is generally considered to be taxonomically remote from the natural order *Rhoadales* (*sensu* Wettstein), comprising most of the traditional isothiocyanate glucoside-containing families such as *Capparidaceae*, *Cruciferae*, *Moringaceae* and *Resedaceae*.

On paper chromatography in several solvent systems, a methanolic extract of disintegrated seed kernels of *P. Roxburghii**** proved to contain at least three mustard oil glucosides (A₁, B and C, Fig. 1), the first two agreeing in their *R_B*-values⁶ with authentic specimens of glucoputranjivin and glucocochlearin, whereas glucoside C appeared at the expected position for a phenyl isothio-

* In a recent study by Bailey *et al.*¹⁴ of the volatile sulphur components in fresh cabbage, phenyl isothiocyanate was tentatively suggested as a trace constituent on basis of an observed peak at mass 135, with isotopic contributions at masses 136 and 137, in mass spectral analyses.

** Freise¹⁵ earlier reported that the latex from *Jatropha multifida* L. (Flor de coral), belonging to the same family, contains a sulphur compound, possibly benzyl isothiocyanate, yet without any experimental documentation.

*** The authors are much indebted to Professor T. R. Govindachari, Presidency College, Madras, India, for the generous gift of the seeds used in the present studies. Seeds can be commercially obtained from United Chemical and Allied Products, 10 Clive Row, Calcutta 1, India.

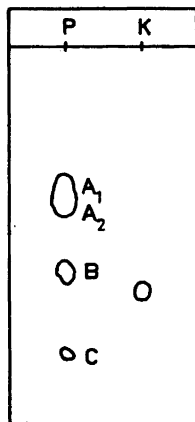


Fig. 1. Paper chromatogram of isothiocyanate glucosides. Solvent system: butanol:ethanol:water (4:1:4). P: Seed extract of *Putranjiva Roxburghii*; K: reference sample of glucotropaeolin. A₁ + A₂: Mixture of glucoputranjivin and glucoceleomin; B: glucochlearin; C: glucojiaputin.

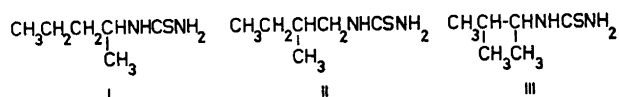
cyanate-producing glucoside. No attempts were made in the present work to isolate, separate and characterize the glucosides.

On enzymic hydrolysis of a methanolic seed extract with a crude, cell-free myrosinase preparation, volatile mustard oils were liberated and removed by steam distillation. Paper chromatography in water-saturated chloroform of the corresponding thiourea derivatives⁷, formed upon reaction with ammonia, revealed strong spots indistinguishable from those of authentic samples of 1-isopropyl- and (+)-1-(2-butyl)-thiourea. At the expected site of 1-phenylthiourea a minute spot appeared, deviating, however, from that of a reference sample by producing an orthodox blue thiourea colour with Grote's reagent rather than the green-blue tint of phenylthiourea. That this thiourea, corresponding to the minor volatile mustard oil derived from glucojiaputin, was not phenylthiourea or any known thiourea of natural derivation became obvious on comparative paper chromatography in other solvent systems (*cf.* Experimental) in which it migrated at a significantly higher rate than phenylthiourea. The first part of this paper deals with the structure elucidation of the new volatile mustard oil.

On a preparative scale, the crude glucoside mixture, extracted from 850 g of defatted seed kernels of *P. Roxburghii* with 70 % methanol, was hydrolyzed in a citrate buffer at pH 6.5 with a crude, cell-free myrosinase preparation. A mixture of the three volatile isothiocyanates was obtained by steam distillation and subsequent ether extraction, and further converted into a mixture of thioureas (15.4 g) on treatment with methanolic ammonia. Upon systematic, fractional crystallization from ethyl acetate, a homogeneous sample of 1-isopropylthiourea (5.2 g) was obtained. Part of the material present in the mother liquors was subsequently subjected to a 155-plate counter-current distribution between chloroform and water, resulting in virtually complete separation of the three thioureas as estimated from paperchromatographic analysis. From the appropriate tubes, an additional amount of 1-isopropylthiourea was isolated whereas the second "band" afforded the *dextrorotatory* (S)-1-(2-butyl)-thiourea, identified on critical comparison with an authentic speci-

men prepared from (+)-2-butyl isothiocyanate. The absolute configuration of the latter was previously established in this laboratory⁸. Contrary to the statement by Puntambekar⁴ that partially racemic 2-butyl isothiocyanate is produced in the enzymic reaction, the preparations obtained in the present work possessed full optical activity.

The unknown, more lipophilic thiourea, completely separated from the isopropyl and 2-butyl derivative, was isolated as colourless prisms in amounts corresponding to about 100 mg per kg of seed kernels. The *dextrorotatory* thiourea, m.p. 72°, possessed the elemental composition C₆H₁₄N₂S, and exhibited ultraviolet and infra-red absorption data compatible with those expected for an N-pentyl-thiourea. The composition, in connexion with the optical activity, in fact limited the possible structures to (I), (II) or (III).



The choice between these was easily made on basis of the n.m.r. spectrum* of the new thiourea (Fig. 2). The presence of six methyl protons and *two* highly deshielded methylene protons (τ 6.8) is compatible only with structure (II).

The absolute configuration of this previously unknown thiourea, and hence of the natural isothiocyanate whence it derives, was established by stereoselective synthesis, departing from *levorotatory* (*S*)-2-methylbutanol (IV)**. According to described or slightly modified procedures, the alcohol was converted into (+)-2-methylbutyl bromide⁹ and this, in turn, into (+)-N-

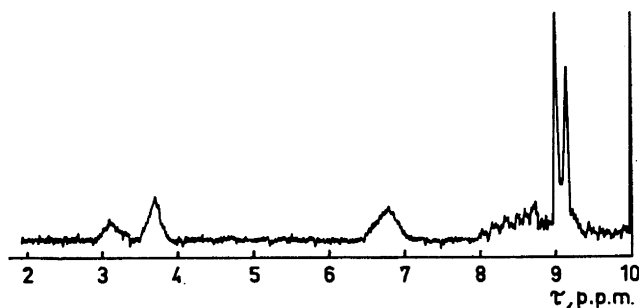
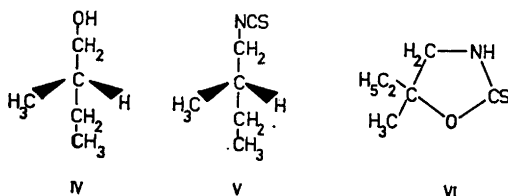


Fig. 2. Proton resonance spectrum (60 Mc) of (+)-1-(2-methylbutyl)-thiourea (II). Solvent: CDCl₃; internal standard: (CH₃)₄Si (τ 10.0).

* The authors are indebted to Dr. A. Melera, Varian A-G., Zürich, for the determination of the n.m.r. spectra.

** The authors are grateful to Dr. E. Egelund Pedersen, The Chemical Laboratory of the University of Copenhagen, for a generous gift of optically pure (-)-2-methylbutanol.

(2-methylbutyl)-phthalimide^{10*}. Hydrazinolysis of the latter afforded (–)-2-methylbutylamine¹⁰, reacting with thiocarbonyl chloride to give the *dextro*-rotatory (*S*)-2-methylbutyl isothiocyanate (V), which was further converted into (+)-1-(2-methylbutyl)-thiourea on reaction with ammonia. The synthetic specimen proved identical with the thiourea of natural derivation in sign and magnitude of rotation as well as with regard to all other physical properties.



Hence, the volatile isothiocyanate produced on enzymic hydrolysis of glucojiaputin is proved to be *dextrorotatory* (*S*)-2-methylbutyl isothiocyanate (V)** and *not*, as stated by Puntambekar⁴, phenyl mustard oil. No trace of the latter isothiocyanate has been observed in enzymically hydrolyzed seed extracts of *P. Roxburghii* in the course of the present work and we can offer no explanation for the literature records^{3,4} of phenyl isothiocyanate as a species of natural derivation.

Gaschromatographic analysis*** of the volatile mustard oil mixture from *Putranjiva* seeds clearly confirmed the presence only of isopropyl, 2-butyl and 2-methylbutyl isothiocyanate. The relative amounts were estimated to be 82 %, 16 % and 2 %, respectively.

A comparative, semi-quantitative evaluation of paper chromatograms of the glucosides (Fig. 1) and the thioureas derivable from the volatile isothiocyanates suggested that an additional glucoside (A_2), inseparable from gluco-putranjivin and affording a non-volatile mustard oil on enzymic hydrolysis, was present in *Putranjiva* seeds. The nature of this glucoside was revealed when a paperchromatographic eluate of the combined glucosides, (A_1) and (A_2), was found to give a blue Grote-colour subsequent to enzymic hydrolysis but without addition of ammonia, and when a characteristic UV-absorption pattern was observed for the Grote-positive compound. These data suggested the presence in the seed extract of a glucosidic 2-oxazolidinethione precursor, analogous

* The rotation and other physical constants of our synthetic specimen of this compound were in excellent agreement with the older data of Marckwald¹⁰. Balenović and Bregant¹¹ recently reported a considerably higher rotation (+ 24°) for a synthetic, liquid preparation giving correct elemental analysis. The reason for this discrepancy is still obscure. Prof. Balenović kindly compared the infra-red spectra of the two preparations and found them practically identical. The physical constants are at present being reinvestigated in Prof. Balenović's laboratory (personal communication).

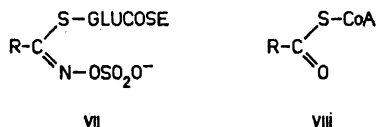
** It may be of biogenetic significance that the configuration of the new isothiocyanate is identical with that prevailing at the C₂-atom of natural L-isoleucine.

*** The authors are indebted to Dr. A. Jart for his assistance in the gaschromatographic analyses.

with those formerly encountered in higher plants (*cf.* Refs. 5,12,13). Consequently, the filtered residue from the large-scale steam distillation was subjected to continuous extraction with ether, resulting in complete transference of the Grote-positive material to the organic solvent. After extraction with aqueous alkali, adjustment to pH 5.5, and renewed continuous ether extraction, a colourless product (2.0 g) was obtained. After purification, this proved identical with (–)-5-ethyl-5-methyl-2-oxazolidinethione (VI) (cleomin), recently reported from this laboratory¹³ as the enzymic hydrolysis product of *gluocleomin*, a glucoside occurring in *Cleome spinosa* Jacq. and several other species of the family *Capparidaceae*. Experiments are in progress to establish the absolute configuration of cleomin.

Puntambekar⁴ reported the fruit pulp and the leaves of *P. Roxburghii* to be devoid of isothiocyanates and glycosidic progenitors. Contrary to this statement, a glucoside pattern, qualitatively and quantitatively similar to that observed in seed extracts, was observed during the present work by paper-chromatographic analyses of fresh leaves, stems and roots*. Ultra-violet spectroscopy further served to demonstrate the presence of gluocleomin in all of the plant parts tested.

No experimental reports have as yet appeared on the biosynthesis of isothiocyanate glucosides (VII). As pure speculation it seems attractive, however, to consider at least some of these as derived *in vivo* from activated esters of the corresponding acids (VIII). The branched side-chains encountered thus



far in this class of glucosides are presented in Fig. 3. It appears that the corresponding acid derivatives (VIII) are identical with or strikingly similar to those involved in the biosynthesis and catabolism of valine, isoleucine and leucine²¹. Thus, isobutyric acid and 3-hydroxyisobutyric acid are believed to be normal intermediates on the degradative pathway of valine in mammalian tissue²² whereas 2-methylbutyric acid, in analogy, arises from isoleucine²³. 2-Ethylhydraacrylic acid may be visualized as resulting from β -oxidation of 2-methylbutyric acid through the shorter chain¹², though such a course admittedly would be at variance with that generally accepted for β -oxidation of α -methyl fatty acids²⁴.

Again, the acid derivatives (VIII) containing a methylene grouping next to the carboxylic function might conceivably originate from partial or complete reduction of the 2,3-dihydroxy acids, functioning as intermediates in the biosynthesis of valine and isoleucine in microorganisms²¹. If so, this course would represent another deviation in higher plants from the enzymic dehydration of the dihydroxy acids in microorganisms to give the α -keto acids whence

* The authors are indebted to the *Botanical Garden of the University of Copenhagen* for the fresh plant material.

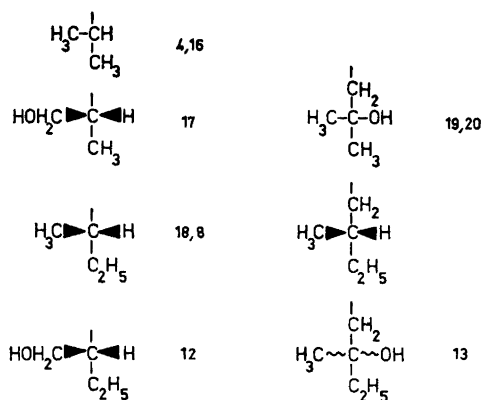


Fig. 3. Known, branched side-chains (R in (VII)) of naturally occurring isothiocyanate glucosides. The figures are literature references.

valine and isoleucine derive²¹. Of course, 3-hydroxyisovaleric acid may equally well be regarded as an established intermediate on the catabolic pathway of leucine²¹, and 3-hydroxy-3-methyl-valeric acid as a reduced form of mevalonic acid.

In this connexion, the recent demonstration by Butler and Butler²⁵ that valine and isoleucine can function as precursors for the cyanogenetic glycosides linamarin and lotaustralin is of interest.

Thus far, isobutyl isothiocyanate has never been encountered as a compound of natural derivation but there may, on basis of biogenetic considerations, be good reasons to expect its future appearance as a product of enzymic glucoside hydrolysis.

EXPERIMENTAL

Melting points are uncorrected and determined in capillary tubes in an Anschütz-Hershberg apparatus. Rotations are measured in a 1 dm tube. Infra-red spectra are determined on a Perkin-Elmer "Infracord"—137 instrument. Analytical samples are dried at room temperature *in vacuo* over calcium chloride.

Paperchromatographic glucoside analysis. A few seed kernels of *P. Roxburghii* were ground in 70 % methanol and the suspension refluxed for 30 min. On paper chromatography in butanol:ethanol:water (4:1:4), followed by spraying with silver-nitrate, the filtrate gave a very strong glucoside spot ($A_1 + A_2$) with an R_B value⁶ of 0.58, a medium spot with R_B 0.90 and a weak spot with R_B 1.25 (Fig. 1). The first two glucosides appeared at exactly the positions of glucopturanjivin and glucochlearin as estimated from controls on the same chromatogram of authentic specimens of these two glucosides, applied in form of an extract of *Cochlearia officinalis* L.¹⁹ Consequently, there can be no doubt that the fast-running component is identical with the glucoside designated *glucojiaputin* by Puntambekar⁴.

Paperchromatographic thiourea analysis. A seed portion (10 g) was crushed in water and the suspension was buffered to pH 6.5 with citrate. A trace of ascorbic acid^{26,25} and a cell-free myrosinase preparation²⁷ (1 ml) were added and the mixture was set aside overnight.

Next day, the suspension was steam-distilled, 0.5 l of distillate being collected in conc. ammonia (15 ml). After 2 h at room temperature, the solution was evaporated to

dryness *in vacuo* and the thiourea mixture (50 mg) was dissolved in ethanol (1.8 ml). On paper chromatography of this solution in water-saturated chloroform⁷, three thiourea spots were observed with R_{Fh} -values⁷ of 0.42, 0.74 and 1.03, the first two being indistinguishable from simultaneously run, authentic samples of isopropyl and 2-butyl thiourea. The very weak spot of the most lipophilic thiourea ran very close to the reference sample of phenylthiourea, yet deviating from this by giving a sky-blue colour with Grote's reagent rather than the green-blue tint of the reference.

Paper chromatography of the same mixture in toluene:ethyl acetate:water (1:1:1) gave the following R_{Fh} -values: 0.58, 0.88 and 1.13, the last value clearly excluding phenylthiourea as a possibility.

Analogously prepared extracts of the thioureas derived from leaves, stems and roots of a fresh plant of *P. Roxburghii* were separately investigated by paper chromatography. Neither the R_{Fh} -values, nor the relative amounts, differed significantly from those observed in the seed extracts.

Isolation and preparation of thioureas. *Putranjiva* seeds (1.5 kg) were suspended in carbon tetrachloride (4.4 l) and disintegrated in a Waring Blender. After filtration, the residue was refluxed twice with fresh 4-l portions of the same solvent, and the defatted seed powder was air-dried (850 g). A glucoside extract was prepared by refluxing the seed powder for 1 h with three 5 l-portions of 70 % methanol. The three filtrates were pooled and concentrated to a small volume *in vacuo*. Water was added to a total volume of 4.2 l, and the solution was filtered through Celite. The enzymic hydrolysis was performed in three 1.4 l-portions. To each were added: water (1.3 l), citrate buffer (150 ml, prepared as follows: 1 M sodium citrate (1 l), citric acid (3.0 g), diluted 20 times with water), myrosinase solution²⁷ (50 ml), and ascorbic acid (25 mg)³⁵. The mixtures were set aside for 17 h at room temperature, the pH-value decreasing during this period from 6.4 to 6.0.

The pooled hydrolysis mixtures were steam-distilled and a total of 6 l of distillate collected. This was then extracted with a total of 4 l of ether. Ammonia-saturated methanol (225 ml) was added to the dried ether solution of the volatile isothiocyanates, and the solution was kept for 40 h at room temperature. Ether was distilled off, and the solid residue (15.4 g) was dissolved in hot water (150 ml), filtered hot from sticky impurities, and the filtrate allowed to cool to give a colourless mixture, (A) (13.6 g), of three thioureas (m.p. 149–157°). The material, (B), contained in the mother liquor (2.5 g, m.p. 128–139°), was processed separately as described below.

(i) *Fraction A.* The thiourea mixture (13.6 g) was recrystallized once from water (90 ml), thrice from ethyl acetate, and finally once again from water to give the predominant thiourea (5.2 g), uncontaminated by other thioureas as established from paper-chromatographic analysis. The pure compound, m.p. 168°, proved to be *1-isopropylthiourea* on comparison with an authentic specimen⁷. Identical infrared spectra and undepressed mixed melting points served to establish the identity.

(ii) *Fraction B.* The above mother liquor fraction (2.5 g) was recrystallized thrice from ethyl acetate to give a mixture (1.13 g) of all three thioureas, as estimated from paper chromatography. The solid was partially dissolved in chloroform (60 ml) and the insoluble part (0.48 g), consisting of only the two major thioureas, was discarded. The chloroform solution was equilibrated with water (60 ml) and the combined volumes introduced into the first 6 tubes of a 60-plate Craig-apparatus, preloaded with water-saturated chloroform. After 155 transfers, with single withdrawal of the aqueous phases after 60 distributions, paperchromatographic analyses indicated a high degree of separation of the three thioureas.

Withdrawn tubes No. 5–19: Evaporation to dryness and recrystallization of the residue afforded an additional amount of *1-isopropylthiourea*, m.p. 170°.

Withdrawn tubes No. 34–65: The solid residue was recrystallized twice from water to give colourless needles, m.p. 134°, $[\alpha]_D^{21} + 27.7^\circ$ (c 3.4, 96 % ethanol), $+ 27.2^\circ$ (c 1.6, CHCl_3). Comparison of rotation values, coinciding infra-red spectra, and undepressed mixed melting point, served to establish the identity of this thiourea with a synthetic specimen of (S)-*1-(2-butyl)-thiourea* described below.

Tubes No. 35–59 in the apparatus: The semi-crystalline residue from these tubes (259 mg) was recrystallized from water (9 ml), with a little charcoal, to give colourless prisms (119 mg) of the unknown thiourea. An analytical specimen was produced on an

additional recrystallization from water, m.p. 72°, $[\alpha]_D^{20} + 7.9^\circ$ (c 1.0, 96 % ethanol) (Found: C 49.13; H 9.68; N 19.08; S 21.90. Calc. for $C_8H_{14}N_2S$: C 49.26; H 9.65; N 19.16; S 21.92). The ultra-violet spectrum in ethanol had the following characteristics: λ_{\max} 242 μ (ϵ 13 670), 204 μ (ϵ 11 300), λ_{\min} 224 μ (ϵ 3 760). The infra-red spectrum (in KBr) exhibited conspicuous bands at: 3 400 (s), 3 220 (vs), 3 080 (m), 2 950 (s), 2 920 (m,sh), 2 880 (m, sh), 1 605 (s), 1 590 (vs), 1 560 (vs), 1 460 (s), 1 430 (s), 1 375 (m), 1 340 (vs), 1 265 (w), 1 225 (w), 1 165 (m), 1 140 (w), 1 015 (w), 970 (w), 940 (w), 890 (w), 770 (w) and 728 (s) cm^{-1} (vs very strong, s strong, m medium, w weak and sh shoulder). The identity of the thiourea as (S)-*I*-(2-methylbutyl)-thiourea appeared from its n.m.r. spectrum (Fig. 2) and was confirmed upon critical comparison with an authentic specimen of the latter, synthesized as described in the sequel.

(S)-2-Butyl isothiocyanate. (S)-2-Butylamine hydrochloride (4.78 g, $[\alpha]_D^{18} - 1.05^\circ$ (c 13.3, H_2O))⁸ was dissolved in 2 N NaOH (21.8 ml), and pure thiocarbonyl chloride (5.0 g) in chloroform (50 ml) was added. Additional 2 N NaOH (44 ml) was added portion-wise, and the chloroform layer was separated after ended reaction. It was washed with HCl (4 N) and water, and the solvent was removed. The remaining (S)-2-butyl isothiocyanate distilled at 47°/11 mm as a colourless liquid (1.6 g), n_D^{20} 1.4940, d_4^{22} 0.9322, $[\alpha]_D^{22} + 66.7^\circ$ (neat). Literature values: $[\alpha]_D + 61.36^\circ$ ³², $[\alpha]_D^{20} + 61.88^\circ$ ³³, $[\alpha]_D + 66.0^\circ$ ²⁸.

(S)-*I*-(2-Butyl)-thiourea. The mustard oil (525 mg) was dissolved in ammonia-saturated methanol (4 ml) and kept at room temperature for a few hours. The solid residue was recrystallized from water to give a pure specimen of (S)-*I*-(2-butyl)-thiourea, m.p. 134°, $[\alpha]_D^{25} + 29.0^\circ$ (c 1.7, $CHCl_3$). Literature values: m.p. 133–134°²⁸, 136–137°¹⁸, $[\alpha]_D^{20} + 22.77^\circ$ (c 3.568, 94 % ethanol)¹⁸, $[\alpha]_D^{20} + 25.65^\circ$ (c 1.65, $CHCl_3$)²⁹, all referring to preparations of natural derivation. It hence seems likely that the previous isolates^{18,29} have been slightly impure or racemized.

(S)-2-Methylbutyl isothiocyanate (V). (S)-2-Methylbutanol ($\alpha_D - 9.49^\circ$ (2 dm tube), n_D^{20} 1.4112) (102 g) was converted into (S)-2-methylbutyl bromide (60.0 g) according to the method of Crombie and Harper⁹, b.p. 120–121°, n_D^{20} 1.4450, d_4^{21} 1.214, $\alpha_D^{23} + 4.90^\circ$ (1 dm), $[\alpha]_D^{20} + 4.04^\circ$ (neat), in good agreement with numerous literature values, e.g. $[\alpha]_D^{20.6} + 4.043^\circ$ ³⁰, n_D^{20} 1.4451³⁰, n_D^{20} 1.4451³¹ *. In chloroform solution our bromide gave the rotation value: $[\alpha]_D^{20} + 4.30^\circ$ (c 4.6) **.

A mixture of the pure bromide (59 g) and potassium phthalimide (79.7 g) in freshly distilled dimethylformamide (250 ml) was stirred, slowly heated to 100°, and kept at this temperature for 3.5 h. The solid was removed and washed with chloroform, the washings being added to the reaction mixture diluted with chloroform (450 ml). The organic phase was subsequently washed with 2 × 150 ml of water, 6 × 100 ml of 0.2 N NaOH and 3 × 150 ml of water. Chloroform and residual dimethylformamide were distilled off from the dried solution whereafter (S)-*N*-(2-methylbutyl)-phthalimide distilled as a colourless oil, b.p. 129–130°/0.06 mm, crystallizing in the receiver, m.p. 25.5° (60 g). (Found: C 71.74; H 7.04; N 6.49. Calc. for $C_{13}H_{16}NO_2$: C 71.84; H 6.96; N 6.45). The following physical constants were determined: n_D^{25} 1.5384, d_4^{22} 1.0972, $[\alpha]_D^{21} + 8.39^\circ$ (neat), $[\alpha]_D^{22} + 8.20^\circ$ (c 0.6, benzene) ***. Literature values¹⁰: m.p. 23°, d_4^{25} 1.0930, $[\alpha]_D^{25} + 7.53^\circ$ (neat).

The substituted phthalimide (58 g) and hydrazine monohydrate (14 g) were dissolved in methanol (500 ml), and the solution was refluxed for 3 h. Water (200 ml) was added, methanol removed *in vacuo*, and the residue was refluxed for 1 h with water (100 ml) and conc. HCl (50 ml). Water (250 ml) was again added to the cooled mixture which was kept overnight in the icebox. Phthalylhydrazide (44 g) was then filtered off and washed

* The value n_D^{20} 1.4552, originally reported by Crombie and Harper⁹, has been corrected in this more recent paper.

** We are inclined to believe that the value $[\alpha]_D^{20} + 5.81^\circ$ (c 4.8, $CHCl_3$), reported by Crombie and Harper⁹, is too high.

*** Balenović and Bregant¹¹ reported $[\alpha]_D^{25} + 24^\circ$ (c 0.56, benzene), cf. foot-note on p. 940.

with ice-cold water, and the combined filtrates were concentrated to dryness *in vacuo*. The amine hydrochloride was redissolved in a small volume of water and solid KOH was added, causing the amine to separate as a yellow top layer. Distillation afforded pure levorotatory (S)-2-methylbutylamine (10 g), b.p. 95°, n_D^{20} 1.4133, d_4^{25} 0.7490, MR₂₅: Found: 28.93, Calc. 28.71; $[\alpha]_D^{25}$ -6.05° (neat). Literature values¹⁰: b.p. 95.5–96°, d_4^{25} 0.7505, $[\alpha]_D^{25}$ -5.86° (neat).

A solution of (-)-2-methylbutylamine (3.25 g) in chloroform (100 ml) was dropwise added to a stirred solution of thiocarbonyl chloride (6 g) in chloroform (50 ml) in the course of 1.2 h. The solution was left overnight at room temperature and the major part of the solvent was removed over a small column. The residue was washed with 2 N HCl (10 ml) and the residual chloroform again removed. Pure dextrorotatory (S)-2-methylbutyl isothiocyanate (V) (1.6 g) distilled as a colourless liquid, b.p. 68.5°/10 mm (Found: C 56.02; H 8.59; N 10.98; S 25.25. Calc. for C₈H₁₁NS: C 55.78; H 8.58; N 10.84; S 24.82). The physical constants were: n_D^{20} 1.4984; d_4^{22} 0.9405; $[\alpha]_D^{22}$ +14.0° (neat, 0.5 dm tube).

Synthesis of (S)-1-(2-methylbutyl)-thiourea. The synthetic mustard oil (250 mg) was dissolved in ammonia-saturated methanol (5 ml) and the solution was left for 24 h at room temperature. The crystalline residue was dissolved in hot water, treated with a little charcoal, and filtered. Colourless needles of (S)-1-(2-methylbutyl)-thiourea separated on cooling (60 mg), m.p. 73°, alone or in admixture with the specimen of natural derivation described above, $[\alpha]_D^{22}$ +7.7° (c 1.04, 96 % ethanol). The synthetic and the naturally derived sample gave coinciding infra-red spectra.

Gaschromatographic analysis. The crude isothiocyanate fraction, obtained by steam distillation of an enzymically hydrolyzed seed extract of *P. Roxburghii*, was subjected to gaschromatographic analysis according to the procedure recently described by Jart³⁴. Squalane served as the stationary phase at a column temperature of 124° and helium as the carrier gas (1.2 l per h).

Isolation of cleomin (VI). The above described enzymically hydrolyzed extract of *Putranjiva* seeds (1.5 kg), from which volatile isothiocyanates had been removed by steam distillation, was filtered through Celite and concentrated *in vacuo* to a volume of 1 l. The aqueous solution was continuously extracted with ether for 36 h, and the ether phase was concentrated to a volume of 500 ml before it was extracted with two 200 ml-portions of 0.2 N NaOH. The ether phase was discarded, and the aqueous solution was adjusted to pH 5.5 with HCl and again extracted continuously with ether for 36 h. The dried ether extract contained an oily residue (6.5 g) that was partly soluble in hot water (115 ml). The solution was treated with charcoal, and the filtrate on cooling deposited colourless needles (317 mg, m.p. 47–48°). A second crop of crystals (2.19 g, m.p. 45–48°) was obtained on concentration of the mother liquor to about 35 ml. A pure specimen was produced on two additional recrystallizations from water, m.p. 52° (Found: C 49.70; H 7.64; N 9.44; S 22.09. Calc. for C₈H₁₁NOS: C 49.63; H 7.64; N 9.65; S 22.08). $[\alpha]_D^{22}$ -26.6° (c 1.06, H₂O). These data, in addition to paperchromatographic analyses, ultraviolet and infra-red spectra, as well as mixed melting point determination, indicated that the isolate was identical with (-)-5-ethyl-5-methyl-2-oxazolidinethione (VI) (cleomin), which was recently established in this laboratory as the enzymic hydrolysis product of glucoleomin, present in *Cleome spinosa* Jacq. and other species of the caper family¹³.

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