ACTA CHEMICA SCANDINAVICA 12 (1958) 1746—1758

*iso*Thiocyanates XXXII.* Synthesis and Reactions of 3-Benzoyloxypropyl *iso*Thiocyanate, Enzymically Liberated from Glucomalcolmiin, and some Compounds of Related Structure

ANDERS KJÆR and ROALD BOE JENSEN

Organic Chemical Laboratory, Royal Veterinary and Agricultural College, Copenhagen, Denmark

Observations resulting in the discovery of a new mustard oil glucoside, *glucomalcolmiin*, in seeds of *Malcolmia maritima* R. Br. *(Cruciferae)* are discussed. Preliminarily reported evidence [1] for the structure of the corresponding *iso*thiocyanate, *malcolmiin*, has been supplemented by unequivocal proof of its structure. 3-Benzoyloxypropyl *iso*thiocyanate has been synthesized and its identity with malcolmiin definitely established. A structural expression for glucomalcolmiin is proposed.

The additional presence in *M. maritima* seeds of glucocheirolin is rigorously proved.

The complex picture observed on ammonia treatment of an enzymically hydrolyzed seed extract of *M. maritima* has been interpreted and the formation of tetrahydro-1,3-oxazine-2-thione corroborated by synthesis. Additional representatives of this little known class of heterocyclic compounds have been synthesized by base-induced ring-closure of the corresponding 3-hydroxyalkyl *iso*thiocyanates. The diminished cyclization tendency of these relative to the 2-hydroxyalkyl mustard oils is pointed out. The biogenesis of glucomalcolmiin is briefly discussed.

A preliminary communication of this series [1] announced the detection in Nature of a new *iso*thiocyanate glucoside, *glucomalcolmiin*, characterized by undergoing enzymic hydrolysis to glucose, sulphate and a mustard oil, tentatively formulated as 3-benzoyloxypropyl *iso*thiocyanate. It is the purpose of the present paper to describe in more detail the experimental data supporting the proposed structure, including the synthesis of the new mustard oil, as well as to expand on the chemistry of some structurally related compounds.

* Part XXXI of this series: *Botan. Tidsskr.* **54** (1958) 36.

The cruciferous genus *Malcolmia* comprises about 25 herbaceous species indigenous to the Mediterranean region. A few of these have attained horticultural significance, *e.g. Malcolmia maritima* (L.) R.Br.* which is easily procurable as seed material from commercial sources **. It is only recently, however, that its contents of *iso*thiocyanate-producing glucosides has been recognized. Thus, an earlier paper of this series [2], surveying the results of paperchromatographic analyses of volatile mustard oils in numerous seed samples, listed *Cheiranthus maritimus* as a species furnishing methyl *iso*thiocyanate on enzymic hydrolysis. This assignment proved untenable when authentic methyl mustard oil glucoside (glucocapparin) was encountered in our laboratory as a natural compound, occurring in several species belonging to the family *Capparidaceae* [3,4]. Schultz and Wagner [5,6], in a communication on paperchromatographic analysis of mustard oil glucosides in numerous plant species, reported the occurrence in seeds of *M. maritima* R.Br. of three partly unidentified glucosides, one of which was found to be present in small amounts only. Essentially the same pattern was observed independently in our laboratory on chromatography of the glucosides in a crude methanolic seed extract [1]. Conspicuous spots, possessing R_B-values [5] of 0.34 and 1.36, were noticed on chromatograms run with the upper layer of the solvent system: *n*-butanol: ethanol: water (4:1:4) as the mobile phase, whereas a minor, and still unidentified glucoside appeared as a weak spot with an R_B-value of 1.03.

Enzymic hydrolysis of a homogeneous preparation of the fastest moving glucoside (R_B 1.36), provided by elution of the appropriate zone from a band paper chromatogram with phosphate buffer, furnished a mustard oil possessing absorption characteristics in the ultra-violet region indicative of aromatic structure. A high-extinction maximum at 232 mμ, together with an auxiliary band of much lower intensity at about 270 mμ, suggested the presence in the *iso*thiocyanate of a conjugated aromatic system, possibly of the benzoyl-type. This pattern, in conjunction with the R_B-value of the parent glucoside, clearly demonstrated this to be different from all heretofore recorded mustard oil glucosides. In accord with common usage within this field the designation *glucomalcolmiin* was therefore introduced for the new natural product [1], affording the corresponding *iso*thiocyanate, *malcolmiin*, on enzymic hydrolysis.

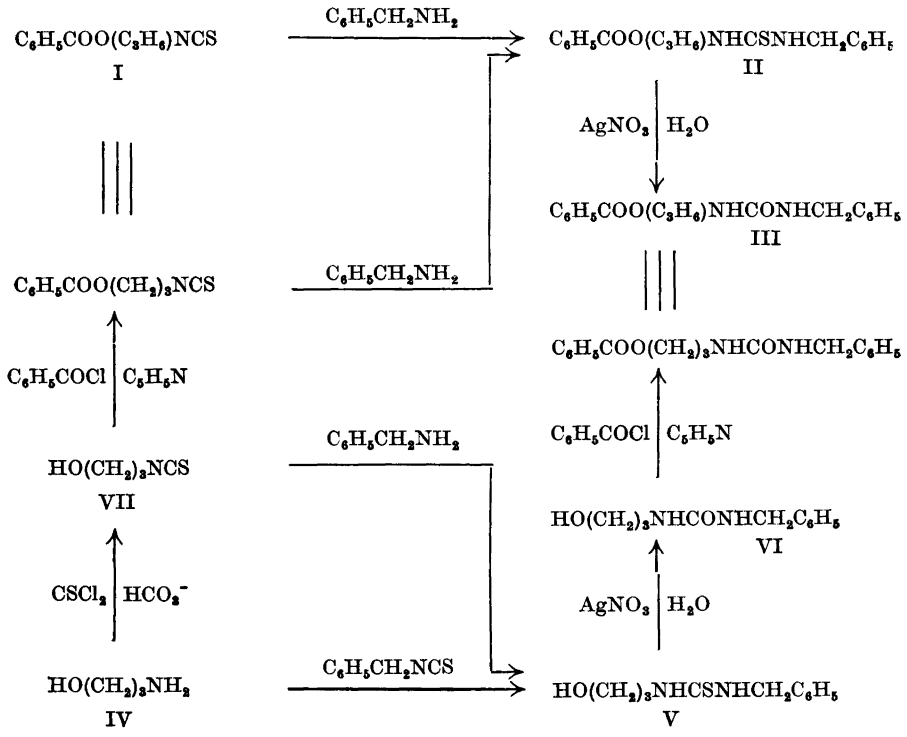
Efforts were first directed towards the isolation of a crystalline thiourea-derivative of malcolmiin. When a purified glucoside extract, prepared from a larger amount of *Malcolmia maritima* seeds, was subjected to enzymic hydrolysis in a buffered solution at pH 6.8, an ether-soluble, optically inactive mustard oil fraction was produced. Subsequent treatment of this with methanolic ammonia resulted in the formation of crystalline malcolmiin-thiourea, $C_{11}H_{14}O_2N_2S$, devoid of optical activity and displaying UV-data in alcoholic solution [1] interpretable as ordinary thiourea-absorption (λ_{max}^{EtOH} 243 mμ) superimposed upon a benzoyl pattern (λ_{max}^{EtOH} 228 mμ, 270—280 mμ). Infrared absorption data [1] provided further evidence for the presence of a benzoate grouping in the thiourea, eventually established beyond doubt by the isola-

* *Nomen conservandum* over *Cheiranthus maritimus* L.

** The seed material employed in the present investigations was obtained from the company *J. E. Ohlsen's Enke*, Copenhagen K., Denmark.

tion of benzoic acid subsequent to alkaline hydrolysis. In water-saturated chloroform malcolmiin-thiourea migrated at a rate corresponding to an R_{Ph}-value [7] of 1.07. Several structural possibilities for malcolmiin, $C_6H_5COO(C_3H_6)NCS$, were discussed in the preliminary paper [1] and evidence adduced in favour of 3-benzoyloxypropyl *iso*thiocyanate, $C_6H_5COO(CH_2)_3NCS$, being the correct formulation.

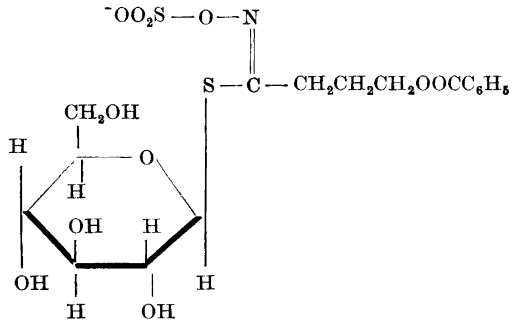
This conclusion has now been verified by various synthetic approaches. Thus, malcolmiin (I) reacted with benzylamine to give the crystalline benzylthiourea (II) which could be smoothly transformed into the corresponding benzylurea (III) on treatment with a solution of silver nitrate in aqueous ethanol. The identity of (III) as 1-(3-benzoyloxypropyl)-3-benzylurea was ascertained by an unequivocal synthesis of the latter starting with 3-aminopropanol (IV).



Reaction of this amino-alcohol with one molecular equivalent of benzyl *iso*thiocyanate afforded 1-benzyl-3-(3-hydroxypropyl)-thiourea (V), smoothly transformed by means of silver nitrate into the corresponding, syrupy benzylurea (VI). When benzoylated in pyridine solution the latter yielded 1-(3-benzoyloxypropyl)-3-benzylurea which proved identical with (III) on critical comparison.

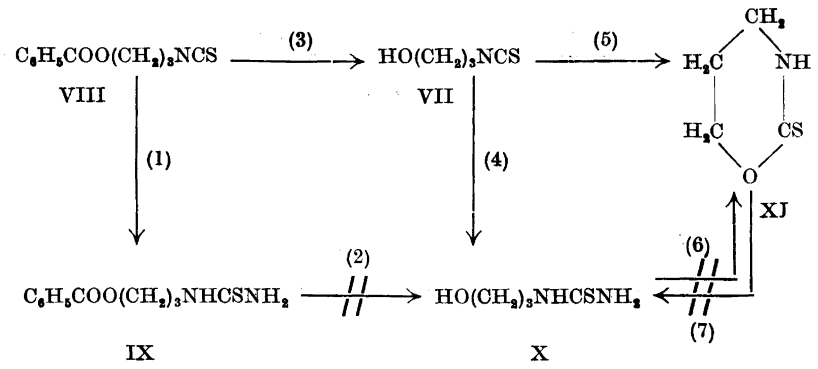
A more direct structure proof for malcolmiin (I) was achieved by its synthesis. After several alternative routes had been unsuccessfully explored it was found that the bifunctional 3-aminopropanol (IV) on treatment with thiocarbonyl chloride in bicarbonate solution afforded a moderate yield of the distillable 3-hydroxypropyl *iso*thiocyanate (VII), the identity of which appeared from its reaction with benzylamine to give a benzylthiourea (V) indistinguishable from the specimen prepared as described above. Benzoylation of (VII) in pyridine solution proceeded easily to give 3-benzoyloxypropyl *iso*thiocyanate as a distillable, colourless oil with correct analytical figures. The identity of the synthetic mustard oil and malcolmiin (I) appeared from the fact that the former reacted with benzylamine to give 1-(3-benzoyloxypropyl)-3-benzylthiourea (II), identical with the benzylthiourea derivative of malcolmiin.

Glucomalcolmiin is a mustard oil glucoside of the ordinary type, furnishing glucose and sulphate in addition to malcolmiin on enzymic hydrolysis. Liberation of sulphate was demonstrated by reaction with barium ions, whereas the formation of glucose was established by paper chromatography in two solvent systems. Again, strong acid treatment of glucomalcolmiin produced hydroxylamine, a characteristic fission product as pointed out by Ettlinger and Lundeen [8] in support of the revised general structure for the *iso*thiocyanate glucosides advanced by these authors. Although conclusive evidence for the stereochemical details are lacking in the present case, it appears very likely that the glucomalcolmiin ion possesses the following structure:



The accessibility of the new mustard oil (I) by synthesis prompted a closer study of its behaviour towards ammonia. The ester linkage and *iso*thiocyanate-grouping both constitute possible sites for nucleophilic attack by the base. In fact, several reaction products were encountered resulting from competing reactions at the vulnerable centers. The following scheme represents the predictable reaction products, the mutual relationships of which were established by paper chromatography.

Upon treatment of synthetic malcolmiin (VIII) with 0.5 N ethanolic ammonia for 40 min. at room temperature, a maximum yield of the corresponding 1-(3-benzoyloxypropyl)-thiourea (IX) was produced together with



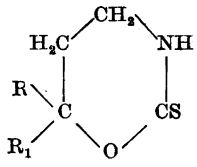
traces of 1-(3-hydroxypropyl)-thiourea (X). Prolonged exposure of (VIII) to ammonia resulted in an increase of the relative amounts of (X), whereas the benzoate-thiourea (IX) proved almost inert towards ammonia under the same conditions. Hence, the route (3)-(4) is the preferred path of reaction rather than (1)-(2) under the present circumstances, indicating an enhanced susceptibility to ammonolysis of the ester mustard oil compared to the ester thiourea. A solution of (VIII), which had been treated for 24 h with ammonia, afforded on paper chromatography in water-saturated chloroform a weak spot with a violet-blue Grote-reaction in addition to those of the thioureas (IX) and (X) and located intermediate between these. The same spot appeared, yet to a much larger extent and supplementary to the expected spot of (X), upon treatment of 3-hydroxypropyl mustard oil (VII) with ammonia. It therefore seemed reasonable to consider the unexpected by-product to be tetrahydro-1,3-oxazine-2-thione (XI), competitively formed by base-induced cyclization of (VII). This assumption was further strengthened by a synthesis of the previously unknown ring-compound (XI)[1] which on paper chromatography afforded a spot indistinguishable from that observed in the ammonia-treated reaction mixture. That the ring compound was produced by the direct route (5) rather than *via* the path (4)-(6) followed from the established inertness of (X) to excess ammonia. Again, the remote possibility of (X) being formed *via* the heterocyclic compound was ruled out on basis of the established stability of (XI) towards ammonia under the conditions employed.

The above conclusions provide a logical interpretation of the complex pattern observed on paper chromatography of crude thiourea fractions of enzymically hydrolyzed seed extracts of *Malcolmia maritima*, as presented and discussed in a preliminary communication [1].

Appropriate processing of a larger seed portion of *M. maritima* disclosed its content of a substantial amount of glucocheirolin, enzymically hydrolyzed to cheirolin (3-methylsulphonylpropyl *iso*thiocyanate) which was characterized as crystalline derivatives formed upon reaction with aniline and benzylamine [1]. The finding of glucocheirolin in *M.maritima* is not surprising in view of the long established occurrence of this glucoside in several *Cheiranthus* species (for a review *cf.* Ref.[9]), closely related botanically to the genus *Malcolmia*. As pointed out previously [1] one of the two spots reported by Schultz and

Wagner [5,6] in thiourea-chromatograms of *M.maritima* extracts was correctly attributed to cheirolin-thiourea whereas the second component, assigned by the same authors to a new, naturally occurring mustard oil, undoubtedly represents 1-(3-hydroxypropyl)-thiourea (X) the artifact character of which in the present case was demonstrated in our preliminary note [1].

The unexpected appearance of the heterocyclic compound (XI) during the present studies rendered a closer investigation of this ring-system desirable. Only one tetrahydro-1,3-oxazine-2-thione seems to have been previously recorded, *viz.* the 4,4,6-trimethyl-derivative prepared by Rosen [10] from 4-amino-4-methyl-2-pentanol. In the course of the current studies (±)-6-methyl- (XII) and 6,6-dimethyl-tetrahydro-1,3-oxazine-2-thione (XIII) were synthesized by treating the appropriate γ-amino-alcohols, *viz.* (±)-4-amino-2-butanol and 4-amino-2-methyl-2-butanol, respectively, with thiocarbonyl chloride in bicarbonate solution to give the corresponding (±)-3-hydroxybutyl (XIV) and 3-methyl-3-hydroxybutyl *iso*thiocyanate (XV) both of which could be cyclized in alkaline solution to the ring compounds (XII) and (XIII), respectively. The distillable mustard oils, (XIV) and (XV), were further characterized as crystalline thioureas formed on reaction with ammonia in chloroform solution. In addition, the benzylthiourea derivative of (XV) was prepared by reaction of 4-amino-2-methyl-2-butanol with benzyl *iso*thiocyanate.

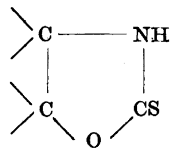


$R = H$, $R_1 = CH_3$: XII
$R = R_1 = CH_3$: XIII

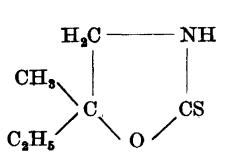
$CH_3CHOHCH_2CH_2NCS$ XIV

$(CH_3)_2C(OH)CH_2CH_2NCS$ XV

In general, it appears that 3-hydroxysubstituted *iso*thiocyanates readily undergo intramolecular cyclization to tetrahydro-1,3-oxazine-2-thiones in alkaline solution, a reaction which is comparable to the analogous and well-established cyclization of 2-hydroxy-alkyl mustard oils to 2-oxazolidinethiones (XVI) [10–15] *. In connexion with the present studies a new representative of the latter class of heterocyclic compounds was synthesized, *viz.* (±)-5-ethyl-5-methyl-2-oxazolidinethione (XVII) derivable from (±)-1-amino-2-methyl-2-butanol which was, in turn, produced by reduction of 2-hydroxy-2-methyl-butyronitrile with lithium aluminium hydride.



XVI



XVII

* For references to earlier papers *cf.* Ref.[11]

Infra-red spectra of the six-membered heterocyclic compounds, (XI)—(XIII), in the solid state or neutral solutions, indicated their character of tetrahydrooxazinethiones rather than dihydrooxazinethiols. In a detailed study, Ettlinger [16] formerly demonstrated several representative compounds of the five-membered class (XVI) to exist entirely in the thione form under comparable conditions.

Contrary to γ-hydroxysubstituted *iso*thiocyanates the tetrahydro-1,3-oxazine-2-thiones, (XI)—(XIII), exert a high-extinction band at 243 mμ in aqueous solution, displaced to 250 mμ in 96 % ethanol. Apart from a minor hyperchromic shift virtually no change of the absorption pattern occurs on addition of alkali provided prolonged exposure to strong base is avoided; if not, a slow but irreversible cleavage of the ring system takes place. Hence, spectrophotometric assays provide a convenient means of following the intramolecular cyclization under varying conditions. From such experiments it was concluded that: (i) a very rapid ring-closure occurs at pH 12 or above in ethanol solution, whereas at pH 10 or below the cyclization is extremely slow, (ii) hydrogen ions are without any promoting effect on the cyclization rate, (iii) a slow ring-closure occurs in boiling ethanol, and (iv) heating to a temperature as high as 125° without solvent does not result in appreciable ring formation. A less striking, but definite promoting effect was demonstrable with triethylamine although paperchromatographic controls indicated that with this base the ring-closure did not proceed to completion. Under standardized conditions there seemed to be a slight increase in the cyclization rate parallel to the increasing number of carbon atoms in the mustard oils. The above observations are consistent with the view that cyclization proceeds by nucleophilic attack of the deprotonized hydroxy substituent on the carbon atom of the *iso*thiocyanate grouping. Hence, the acid strength of the hydroxy-group as well as the intrinsic cyclization tendency may be expected to influence the over-all reaction rate, which, however, appears to be generally less than that prevailing in the corresponding 2-hydroxysubstituted *iso*thiocyanates under comparable conditions. Thus far, all syntheses designed to yield the latter compounds have invariably resulted in cyclized products.

Although no experimental evidence is yet available it appears reasonable to envisage glucomalcolmiin as biogenetically derived from *des*benzoylglucomalcolmiin containing the 3-hydroxypropyl-side chain. Furthermore, it may be of interest to consider a possible generic relationship of the latter with other well-established glucosides containing a 3-carbon unit in their side-chains, such as sinigrin, with the allyl-grouping, and glucoibervirin [17] possessing the methylthiopropyl radical as its side-chain. Glucoiberin and glucocheirolin may tentatively be regarded as secondarily formed oxidation products of glucoibervirin. Several examples of enzymic transformations between analogously substituted compounds can be adduced from modern biochemistry and suggest a common biogenetic pathway for these glucosides, a possibility which will be submitted to experimental trial in forthcoming studies.

EXPERIMENTAL

Melting points are uncorrected and determined in capillary tubes in a slowly heated bath. Analytical specimens have been dried *in vacuo* over calcium chloride at room temperature.

Most of the synthetic compounds described in the sequel have been prepared with no special view to establishing the optimal conditions for the reactions involved. Hence, in many cases the reported yields most likely can be improved considerably.

Spectroscopic detection of glucomalcolmiin. A concentrated methanolic extract of finely ground seeds of *Malcolmia maritima* was applied as a narrow band to the starting line of a 15 cm broad strip of prewashed Whatman paper No. 1. After descending chromatography for 24 h with the upper layer of the system *n*-BuOH:EtOH:H_2O (4:1:4) as the mobile phase, two edge cuts were employed for locating the band of the fastest moving glucoside by means of a silver nitrate spray. The appropriate area of the paper was cut out, eluted with dilute phosphate buffer (pH 6.8), and the resulting solution subjected to enzymic hydrolysis on addition of a drop of a cell-free myrosinase preparation [18]. After extraction of the liberated mustard oil (malcolmiin) with ether and displacement of the solvent by water, the ultra-violet absorption spectrum was determined. A sharp peak at 232 mμ and an inflexion of much lower extinction near 275 mμ were characteristic features, suggesting aromatic character of malcolmiin.

Isolation of malcolmiin and cheirolin as thiourea-derivatives. Finely ground seeds (500 g) of *M. maritima* were defatted with a total of 4 l of boiling carbon tetrachloride and then thoroughly extracted with 70 % methanol (2.5 l). After removal of the solvent *in vacuo*, the residue was taken up in water, treated with excess lead acetate and the white precipitate filtered off and discarded. Lead ions in the filtrate were precipitated by hydrogen sulphide and after repeated filtrations the solution was buffered with phosphates to pH 6.8, adjusted to a total volume of 2 l with water and subjected to enzymic hydrolysis by addition of a cell-free myrosinase preparation (15 ml). At the end of 12 h the mixture was extracted, first with three 300-ml portions of ether and then thrice with the same volumes of chloroform. In order to remove some coloured impurities both extracts were briefly washed with cold 1 N NaOH and then with water. The dried and concentrated extracts were now processed separately.

The chloroform fraction was treated with benzylamine (1 ml), resulting in the separation of a crystalline product (470 mg). Two recrystallizations from 10 % ethanol afforded *1-benzyl-3-(3-methylsulphonylpropyl)-thiourea* (cheirolin-benzylthiourea) (390 mg) as colourless, rhombic plates, m. p. 116°, identified on comparison with an authentic, synthetic specimen (m. p. 116°), prepared from cheirolin [19] and benzylamine (analyses previously published [1]).

The ether extract was divided into two halves. To the first was added aniline (1 ml), and the resulting crystalline precipitate (84 mg) was recrystallized twice from water to yield *1-(3-methylsulphonylpropyl)-3-phenylthiourea* (cheirolin-phenylthiourea) (54 mg), m. p. 138°, undepressed on admixture with a previously prepared synthetic specimen [19].

The remaining half of the ether extract was kept overnight in ammonia-containing methanol. Next day, the solvent was removed and the residue treated with hot ethyl acetate, leaving a slight amount of cheirolin-thiourea undissolved. Concentration of the filtrate resulted in separation of an oily fraction, paperchromatographically proved to be a mixture of 3-hydroxypropylthiourea (X) and tetrahydro-1,3-oxazine-2-thione (XI). Further concentration of the supernatant yielded a solid product which proved homogeneous on paper chromatography after one recrystallization from aqueous methanol (78 mg), m. p. 159°. Additional recrystallizations, first from ethyl acetate and then from 30 % ethanol, afforded *malcolmiin-thiourea* (23 mg) as colourless, dense, optically inactive crystals, m. p. 162° (analyses, UV- and IR-data as well as the R_{Ph}-value have been previously published [1]), undepressed on admixture with a specimen of synthetic 1-(3-benzoyloxypropyl)-thiourea (IX) described in the following.

A separate preparation of an ether extract, procured as just described, was treated with benzylamine to yield crystalline *malcolmiin-benzylthiourea* (101 mg), separating from a mixture of ethyl acetate and hexane as colourless crystals, m. p. 80.5°. Mixed melting point determination and coinciding infra-red spectra served to establish the identity of this derivative with a synthetic sample of 1-benzyl-3-(3-benzoyloxypropyl)-thiourea described in the sequel.

Desulphurization of malcolmiin-benzylthiourea (II). The benzylthiourea of natural malcolmiin (II) (100 mg), dissolved in 90 % ethanol (2 ml), was treated with silver nitrate (214 mg) in water (2 ml), and the solution was heated on a water-bath for 0.5 h. pH was kept at 7 by gradual addition of 0.5 N NaOH during the heating process. Precipitated Ag_2S was filtered off and the reaction product (15 mg) caused to separate by addition of water. Three recrystallizations from 80 % ethanol yielded a pure specimen (3 mg) of a compound, m. p. 145°, identified as 1-(3-benzoyloxypropyl)-3-benzyl-urea (III). No depression of the melting point was observed on admixture with an authentic specimen of the latter, prepared as described below. Furthermore, coinciding infra-red spectra served to confirm the identity.

Degradation of malcolmiin-thiourea. A sample of partly purified malcolmiin-thiourea (73 mg), secured as described above, was dissolved in ethanol containing excess of 0.1 N NaOH and left at room temperature for 48 h. The acidified solution was then extracted with ether and the extract concentrated to dryness. The residue (40 mg) was treated with charcoal in hot hexane, recrystallized once from water and finally sublimed to give pure benzoic acid (17 mg), m. p. 123°, undepressed on admixture with an authentic sample.

The aqueous solution was concentrated to dryness, dissolved in a few drops of water and chromatographed on paper, both in water-saturated chloroform and *n*-butanol: ethanol:water, together with synthetic 3-hydroxypropylthiourea *(vide infra)* from which it could not be distinguished. Both remained on the starting line in chloroform but travelled corresponding to an R_F-value of *ca.* 0.52 in the alcoholic system.

Degradation of glucomalcolmiin. A glucomalcolmiin fraction, prepared by elution of the appropriate zones from several band chromatograms, was subjected to enzymic cleavage in the usual way. After removal of malcolmiin by ether extraction, the concentrated aqueous phase was assayed for glucose by paper chromatography in *n*-butanol-ethanol:water (4:1:4) and pyridine:amyl alcohol:water (35:30:30). R_F-values of 0.16 and 0.34 were determined, respectively, the same as for simultaneously run reference samples of authentic D-glucose.

A separate sample of the aqueous phase yielded a positive sulphate reaction when tested with barium chloride. In both cases, appropriate blanks were checked in order to exclude positive reactions from possible trace constituents of the paper or enzyme preparation.

The dry residue from a similarly prepared solution of glucomalcolmiin was left overnight with excess of conc. HCl at room temperature. The liberation of hydroxylamine was demonstrated paperchromatographically by the method employed by Ettlinger and Lundeen [8].

1-Benzyl-3-(3-hydroxypropyl)-thiourea (V). 3-Aminopropanol (1.0 g) and benzyl *iso*thiocyanate (2.0 g) were mixed in ether (25 ml) and left at room temperature for 24 h. After removal of the solvent, the crystalline product was recrystallized twice from aqueous ethanol to yield the pure thiourea (2.84 g), m. p. 99° (Found: C 58.90; H 7.06; N 12.41. Calc. for $C_{11}H_{16}N_2OS$: C 58.89; H 7.19; N 12.49).

1-Benzyl-3-(3-hydroxypropyl)-urea (VI). The above thiourea (V) (2.0 g) was dissolved in ethanol (35 ml) and a solution of silver nitrate (3.0 g) in water (5 ml) was added. The mixture was heated on a water-bath for 20 min., and 0.5 N NaOH was gradually added to maintain pH 7. The solvents were removed in vacuum after filtration, and the reaction product dissolved in abs. ethanol and thus separated from $NaNO_3$. On concentration a colourless syrup separated which showed no signs of crystallization by various treatments.

1-(3-Benzoyloxypropyl)-3-benzyl-urea (III). To a solution of the syrupy urea (VI) (1.76 g) in dry pyridine (20 ml) freshly distilled benzoyl chloride (1.30 g) was added, and the mixture was set aside for 24 h. The brown solution was then diluted with ice-water (50 ml) when the oily precipitate readily crystallized. Three recrystallizations from 80 % ethanol afforded a pure specimen (686 mg) of the benzoylurea as glistening needles, m. p. 146°, alone or in admixture with the preparation above, derived from 'natural' malcolmiin-benzylthiourea. (Found: C 69.10; H 6.60; N 8.89. Calc. for $C_{18}H_{20}N_2O_3$: C 69.20; H 6.45; N 8.97). The infra-red spectrum (in KBr) displayed a strong benzoate C=O-band at 1 722 cm^{-1}.

3-Hydroxypropyl iso*thiocyanate* (VII). A mixture of 3-aminopropanol (16 g) in 5 % $NaHCO_3$-solution (270 ml) and thiocarbonyl chloride (25 g) in chloroform (350 ml) was vigorously stirred until the thiophosgene colour had disappeared. The dried chloroform

solution was concentrated and the yellow, pungent oil was distilled *in vacuo*. A small fore-run was followed by a constant boiling fraction (6.5 g), b. p. 80°/2 mm, of almost colourless 3-hydroxypropyl mustard oil, n_D^{25} 1.5432. A center-cut sample was analyzed. (Found: C 41.20; H 6.08; N 11.90. Calc. for C_4H_7NOS: C 40.98; H 6.02; N 11.95). A residue (2—3 g) of a very viscous product remained in the distilling flask. After a few weeks a distilled sample had changed into a similar product, probably of polymeric nature (polythiourethanes).

In order to prove the identity of the distilled mustard oil, a small sample was allowed to react with benzylamine in ether to give a quantitative yield of 1-benzyl-3-(3-hydroxypropyl)-thiourea (V) which, recrystallized from aqueous ethanol, had the m. p. 99°, undepressed on admixture with the product described above, prepared from 3-aminopropanol and benzyl *iso*thiocyanate.

3-Benzoyloxypropyl iso*thiocyanate (malcolmiin)* (I). In a typical experiment freshly distilled 3-hydroxypropyl mustard oil (1.56 g) was dissolved in dry pyridine (7 ml) and freshly distilled benzoyl chloride (2.5 g) was added. After 14 h at room temperature excess dilute HCl was added. When all benzoyl chloride was hydrolyzed, the solution was extracted with ether. The organic layer was washed with 5 % $NaHCO_3$-solution and water, and dried. The new *iso*thiocyanate distilled as a colourless oil (1.20 g), b. p. 144°/0.4 mm, n_D^{25} 1.5687. (Found: C 59.60; H 4.93; N 6.43. Calc. for $C_{11}H_{11}NO_2S$: C 59.73; H 5.01; N 6.33.)

1-(3-Benzoyloxypropyl)-thiourea (IX). Synthetic malcolmiin (150 mg) was dissolved in abs. ethanol (10 ml) and a 6 N solution of ammonia in the same solvent (1 ml) was added. After 40 min. at room temperature, the solvent and excess ammonia was removed *in vacuo*, leaving the thiourea (156 mg) as a colourless solid. This was recrystallized once from 30 % ethanol and once from ethyl acetate to give pure malcolmiin-thiourea (76 mg), m. p. 162°. Mixed melting point determination and coinciding infra-red spectra served to establish the identity of the product with the thiourea prepared from 'natural' malcolmiin and described above.

1-(3-Benzoyloxypropyl)-3-benzyl-thiourea (II). When synthetic malcolmiin (150 mg) was mixed with benzylamine (150 mg) in ether (5 ml), and the solution left overnight at room temperature, (II) (210 mg) crystallized on removal of the solvent and trituration of the residue with a few drops of dilute acid. Recrystallization, first from aqueous ethanol and thence from a mixture of ethyl acetate and hexane, afforded a colourless specimen for analysis, m. p. 80.5°, alone or in admixture with the above preparation from 'natural' malcolmiin. (Found: C 65.80; H 6.10; N 8.39. Calc. for $C_{18}H_{20}N_2O_2S$: C 65.82; H 6.14; N 8.53.)

1-(3-Hydroxypropyl)-thiourea (X). A solution of freshly distilled 3-hydroxypropyl *iso*thiocyanate (VII) (600 mg) in chloroform (100 ml), saturated with dry ammonia, deposited after 12 h at room temperature a crop (572 mg) of colourless crystals. An analytical specimen was produced by three recrystallizations from nitromethane, m. p. 131°. (Found: C 35.70; H 7.47; N 20.82. Calc. for $C_4H_{10}N_2OS$: C 35.80; H 7.51; N 20.89.) The thiourea (X) travels on paper chromatography in *n*-butanol-ethanol:water (4:1:4) at a rate corresponding to an R_F-value of 0.52.

Tetrahydro-1,3-oxazine-2-thione (XI). 3-Aminopropanol was subjected to mustard oil synthesis under non-aqueous conditions, according to the directions given by Hodgkins and Ettlinger [20]. The solution of the reaction product in triethylamine-containing chloroform was concentrated to a small volume, acidified with HCl and thoroughly extracted with ether. The turbid, dry ether extract contained a greenish oil which was refluxed in benzene solution for 0.5 h. Evaporation of the solvent resulted in the separation of a partly crystalline product which was purified by charcoal treatment in chloroform solution. Two recrystallizations from chloroform-pentane mixtures yielded the pure ring-compound as colourless plates, m. p. 129°. (Analyses previously published [1].) Yields were of the order of 20 % but could undoubtedly be improved. The ultra-violet spectrum in 96 % ethanol had λ_{max} 250 mμ (ε 14 200), in water: λ_{max} 243 mμ (ε 13 700). Addition of NaOH resulted in an increased molecular extinction (ε 16 300), yet at the same wavelength.

The infra-red spectrum (in KBr), though reminiscent of that of 2-oxazolidinethione, possessed several characteristic, individual features. Conspicuous bands were observed

at: 765 (m, broad), 810 (s), 914 (s), 964 (s), 1 052 (vs), 1 130 (s), 1 168 (vs), 1 210 (m), 1 238 (vs), 1 315 (vs), 1 374 (m), 1 478 (m), 1 578 (vs) and 3 200 (s) cm^{-1}.

In water-saturated chloroform the heterocyclic compound travelled at a rate corresponding to an R_{Ph}-value of 0.78.

(±)-3-Hydroxybutyronitrile. Propylene chlorohydrin (200 g), prepared from propylene oxide and HCl[21], was refluxed for 5 h with slightly more than one molecular equivalent of KCN dissolved in ethanol, and a trace of KI. The solvent was then distilled off and the residue fractionated *in vacuo* to give racemic 3-hydroxybutyronitrile (185 g) as a colourless liquid, b. p. 103°/13 mm. A similar procedure has been previously employed by Bruylants and Castille [22].

(±)-4-Amino-2-butanol. This amino-alcohol has previously been synthesized either by aluminium amalgam reduction of 2-hydroxybutyraldoxime [23,24] or, in 48 % over-all yield, by a two-step process from 1,3-butanediol cyclic sulphate [25]. In the present work, however, reduction of 3-hydroxybutyronitrile (8.5 g) with lithium aluminium hydride (10 g) in dry ether (300 ml), carried out in the usual way, proceeded satisfactorily to give 5.4 g of distilled amino-alcohol, b. p. 74—82°/10 mm. The broad boiling point range, noticed also by other workers [25], is undoubtedly ascribable to hydrate formation. The picrate melted at 122° (literature values [23,25] 122°.)

(±)-3-Hydroxybutyl iso*thiocyanate* (XIV). The above amine (3.6 g), dissolved in saturated $NaHCO_3$-solution (150 ml), was vigorously stirred with a solution of thiocarbonyl chloride (4.73 g) in chloroform (100 ml), until the red colour had disappeared. The chloroform layer was washed with dilute HCl and water, dried and distilled to remove the solvent. The residual oil was fractionated *in vacuo* to give a pure preparation (1.08 g) of the almost colourless mustard oil, b. p. 89°/0.5 mm, n_D^{25} 1.5267. (Found: C 45.95; H 6.96; N 10.50. Calc. for C_5H_9NOS: C 45.77; H 6.92; N 10.68.)

(±)-1-(3-Hydroxybutyl)-thiourea. A solution of the mustard oil (XIV) (150 mg) was kept in ammonia-saturated chloroform (75 ml) for 12 h. Removal of the solvent left a syrup which crystallized in contact with nitromethane to give the colourless thiourea (37 mg), which was recrystallized from the same solvent before analysis, m. p. 115°. (Found: C 40.49; H 8.09; N 18.80. Calc. for $C_5H_{12}N_2OS$: C 40.53. H 8.17; N 18.91.) In *n*-butanol-ethanol:water (4:1:4) the thiourea travels with an R_F-value of 0.64.

(±)-6-Methyl-tetrahydro-1,3-oxazine-2-thione (XII). A chloroform solution of 3-hydroxybutyl mustard oil, prepared from 3.78 g of 4-amino-2-butanol, as described above, was stirred with 1 N NaOH for 2 h at room temperature. After acidification, the organic phase was separated, dried and concentrated to yield a crystalline crop, embedded in an oil. The latter was removed by ether treatment and subsequently demonstrated to consist essentially of non-cyclized mustard oil.

The crystalline fraction (1.15 g) was purified by repeated recrystallizations from chloroform-pentane mixtures to give colourless needles, m. p. 142°. (Found: C 45.55; H 6.85; N 10.50. Calc. for C_5H_9NOS: C 45.77; H 6.92; N 10.68.) λ_{max}^{EtOH} 250 mμ (ε 15 500). The ring-compound travels in chloroform with an R_F-value of 0.82 (R_{Ph} 1.05).

3-Hydroxy-3-methyl-butyronitrile. 1-Chloro-2-methyl-2-propanol [26] (33 g) was dissolved in ethanol and placed together with excess NaCN in a round-bottomed flask, equipped with a reflux condenser, and heated locally to start an exothermic reaction. After this had ceased the mixture was gently refluxed for 1 h. The solvent was then removed, water was added and the reaction products extracted with ether. Distillation gave the pure cyanohydrin (21 g), b. p. 114°/30 mm, n_D^{25} 1.4277. (Found: C 60.80; H 9.08; N 13.90. Calc. for C_5H_9NO: C 60.59; H 9.15; N 14.14.)

The hydroxynitrile has been previously reported, yet with a deviating b. p., *viz.* 130—132°/30 mm (n_D 1.42911) [27].

4-Amino-2-methyl-2-butanol. Reduction of the above hydroxynitrile (10 g) with $LiAlH_4$ (7.6 g) in the usual way gave, after two distillations, the colourless amine as a viscous oil (5.3 g), b. p. 71.5°/10 mm, n_D^{25} 1.4484. (Found: C 58.20; H 12.65; N 13.63. Calc. for $C_5H_{13}NO$: C 58.19; H 12.70; N 13.58.)

A poorly characterized specimen of the same amine was reported by Barger and White [28] as a hydrolysis product of hydroxydihydrogalegin and also as a product resulting from the reaction between 4-amino-2-methyl-2-butene and dilute sulphuric acid.

1-Benzyl-3-(3-hydroxy-3-methyl-butyl)-thiourea. A solution of the above amine (300 mg) and benzyl *iso*thiocyanate (420 mg) in dry ether (20 ml) deposited after 0.5 h at room temperature a crop (594 mg) of almost pure thiourea. An analytical specimen was secured by two recrystallizations from aqueous ethanol, m. p. 127°. (Found: C 61.93; H 7.86; N 10.92. Calc. for $C_{13}H_{20}N_2OS$: C 61.86; H 7.99; N 11.05.)

3-Hydroxy-3-methyl-butyl iso*thiocyanate* (XV). 4-Amino-2-methyl-2-butanol (3.10 g) was treated with thiocarbonyl chloride (3.45 g) in bicarbonate solution exactly as described above for the lower homologue, to give 1.73 g of redistilled mustard oil, b. p. 77°/0.3 mm, n_D^{25} 1.5220. (Found: C 49.70; H 7.49; N 9.58. Calc. for $C_6H_{11}NOS$: C 49.63; H 7.64; N 9.65.)

1-(3-Hydroxy-3-methyl-butyl)-thiourea. This derivative was prepared from the above mustard oil (200 mg) and ammonia-saturated chloroform as described above for the lower homologue. The pure thiourea (150 mg) was obtained after two recrystallizations from nitromethane, m. p. 99°. (Found: C 44.54; H 8.60; N 17.14. Calc. for $C_6H_{14}N_2OS$: C 44.39; H 8.69; N 17.26.) On paper chromatography in *n*-butanol:ethanol:water (4:1:4) the R_F-value 0.69 was determined.

6,6-Dimethyl-tetrahydro-1,3-oxazine-2-thione (XIII). When 3-hydroxy-3-methyl-butyl *iso*thiocyanate (200 mg) was treated for 15 min. in ethanol (2 ml) with 0.1 N NaOH (15 ml) and then brought to pH 7 with acid, ether extraction yielded the cyclized product in crystalline form. An analytical specimen (40 mg) separated from ethyl acetate-hexane mixtures as colourless, nacreous plates, m. p. 136°. (Found: C 49.56; H 7.69; N 9.56. Calc. for $C_6H_{11}NOS$: C 49.63; H 7.64; N 9.65.) λ_{max}^{EtOH} 251 mμ (ε 14 500). R_F in chloroform 0.90 (R_{Ph} 1.15).

(±)-1-Amino-2-methyl-2-butanol. Methyl ethyl ketone cyanohydrin (7.6 g) was smoothly reduced with lithium aluminium hydride (5.3 g) in anhydrous ether (200 ml) under the usual conditions. The resulting amino-alcohol was obtained after distillation as a viscous, colourless oil (3.2 g), b. p. 69°/12 mm. It was characterized as the *neutral oxalate*, separating from hot ethanol in nacreous plates, m. p. 193°. (Found: C 48.58; H 9.50; N 9.43. Calc. for 2 $C_5H_{13}ON$, $C_2H_2O_4$: C 48.62; H 9.52; N 9.45.)

The amino-alcohol has formerly been characterized as its nitrate [30] and its hygroscopic hydrochloride, the latter though with varying melting points [29,30].

(±)-5-Ethyl-5-methyl-2-oxazolidinethione (XVII). The above amine (2.5 g) was subjected to the general conditions designed by Hodgkins and Ettlinger [20] for mustard oil synthesis under non-aqueous conditions. The oily reaction product crystallized in a desiccator to the cyclic compound (1.20 g) of which an analytical specimen separated slowly from water as compact, colourless rhombs, m. p. 52–53°. (Found: C 49.15; H 7.57; N 9.58. Calc. for $C_6H_{11}NOS$: C 49.63; H 7.64; N 9.65.)

The valuable assistance of Dr. R. Gmelin in the preliminary phases of the present work is gratefully acknowledged. Microanalyses have been performed by Mr. P. Hansen at the Chemical Laboratory of the University of Copenhagen.

The work is part of investigations supported by *Statens Almindelige Videnskabsfond (The Danish State Research Foundation)* and *Carlsbergfondet (The Carlsberg Foundation)*. A special grant from *Kai Hansen's Fond* is also gratefully acknowledged.

REFERENCES

1. Kjær, A. and Gmelin, R. *Acta Chem. Scand.* **10** (1956) 1193.
2. Kjær, A., Conti, J. and Larsen, I. *Acta Chem. Scand.* **7** (1953) 1276.
3. Kjær, A., Gmelin, R. and Larsen, I. *Acta Chem. Scand.* **9** (1955) 857.
4. Kjær, A. and Gmelin, R. *Acta Chem. Scand.* **10** (1956) 335.
5. Schultz, O.-E. and Wagner, W. *Z. Naturforsch.* **11b** (1956) 73.
6. Wagner, W. *Papierchromatographische Analyse der Senfölglucoside, präparative Darstellung ihrer Acetylderivate und ein Beitrag zu ihrer allgemeinen Struktur* (Diss.) University, Tübingen 1956.
7. Kjær, A. and Rubinstein, K. *Acta Chem. Scand.* **7** (1953) 528.
8. Ettlinger, M. G. and Lundeen, A. J. *J. Am. Chem. Soc.* **78** (1956) 4172.

9. Kjær, A. *Encyclopedia of Plant Physiology*, Springer-Verlag, Berlin-Göttingen-Heidelberg, 1958, vol. IX, p. 71.
10. Rosen, A. A. *J. Am. Chem. Soc.* **74** (1952) 2994.
11. Kjær, A., Gmelin, R. and Boe Jensen, R. *Acta Chem. Scand.* **10** (1956) 432.
12. Ettlinger, M. G. *J. Am. Chem. Soc.* **72** (1950) 4792.
13. Viscontini, M. and Adank, K. *Helv. Chim. Acta* **33** (1950) 2251.
14. Bergmann, E. D. and Herman, D. F. *J. Appl. Chem. (London)* **3** (1953) 42.
15. Kjær, A. and Gmelin, R. *Acta Chem. Scand.* **11** (1957) 906.
16. Ettlinger, M. G. *J. Am. Chem. Soc.* **72** (1950) 4699.
17. Kjær, A., Gmelin, R. and Larsen, I. *Acta Chem. Scand.* **9** (1955) 1143.
18. Neuberg, C. and Wagner, J. *Biochem. Z.* **174** (1926) 457.
19. Kjær, A., Marcus, F. and Conti, J. *Acta Chem. Scand.* **7** (1953) 1370.
20. Hodgkins, J. E. and Ettlinger, M. G. *J. Org. Chem.* **21** (1956) 404.
21. Michael, A. *Ber.* **39** (1906) 2785.
22. Bruylants, P. and Castille, A. *Bull. soc. chim. Belges* **34** (1925) 261.
23. Robinson, R. and Suginome, H. *J. Chem. Soc.* **1932** 304.
24. Sasaki, S. and Ri, S. *Bul. Sci. Fak. Terkult. Kyushu Imp. Univ. Fukuoka, Japan* **11** (1944) 86; *cf. Chem. Abstracts* **43** (1949) 5367.
25. Lichtenberger, J. and Lichtenberger, R. *Bull. soc. chim. France* **1948** 1002.
26. Burgin, J., Hearne, G. and Rust, F. *Ind. Eng. Chem.* **33** (1941) 385.
27. Lemaire, J. *Rec. trav. chim.* **29** (1910) 22, p. 59.
28. Barger, G. and White, F. D. *Biochem. J.* **17** (1923) 827.
29. Fourneau, E. *J. pharm. chim.* [7] **2** (1910) 56, 109; *cf. Chem. Zentr. 1910*, II, 1365.
30. Barbière, J. *Bull. soc. chim. France* **11** (1944) 470.

Received July 5, 1958.