3-Methyl-3-butenylglucosinolate, a New Isothiocyanate-Producing Thioglucoside*

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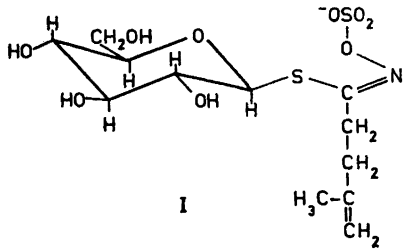
During current studies of isothiocyanate-producing glucosides in species of the family Capparidaceae it was found that leaves of *Capparis linearis* Jacq. contain a new compound of this class for which we now report the structure (I).

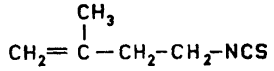


I

C. linearis Jacq. is a small tree or shrub indigenous to the arid zones of Colombia and Venezuela. Paper chromatography of a 70 % methanolic extract of dried leaf material disclosed its content of only one thioglucoside. Enzymic hydrolysis of the latter with myrosinase afforded, in addition to glucose and sulphate ions, a volatile isothiocyanate, convertible into a chromatographically homogeneous thiourea on treatment with ammonia.

Acetylation of a purified, amorphous potassium salt of the glucoside, produced by ion-exchange technique, afforded a tetraacetate which crystallized as the slightly levorotatory tetramethylammonium salt, $C_{24}H_{40}O_{13}N_2S_2$, $0.5H_2O$. Acid hydrolysis of the latter yielded, *inter alia*, hydroxylamine. Hence, the new glucoside is of the usual type [1] and possesses a C_5H_9-side-chain.**

The corresponding isothiocyanate, liberated enzymically from the amorphous glucoside fraction, was isolated by steam-distillation and further purified by preparative gas chromatography. Its composition, C_5H_9NCS, agreed with the above deduction, and its structure was easily revealed by spectroscopic methods. The mass-spectrum exhibited, in addition to the molecular ion (*m/e* 127), strong peaks at *m/e* 112 (M−15), *m/e* 72 (base peak) (CH_2NCS^+),[3] *m/e* 55 ($C_4H_7^+$), and *m/e* 41 ($C_3H_5^+$). This fragmentation pattern strongly suggested the structure (II) for



II

the new isothiocyanate. The NMR-spectrum (in $CDCl_3$) fully confirmed this conclusion and displayed the expected signals: δ 1.76 ppm (3H) (CH_3, singlet, slightly split by the vinylic protons), δ 2.38 ppm (2H) ($C-CH_2-C$, triplet, with slight secondary splitting from the vinylic protons), δ 3.62 ppm (2H) ($C-CH_2-NCS$, triplet), and δ 4.82 ppm (2H) ($H_2C=C-$, doublet, with secondary splitting). Infra-red data were likewise in accordance with the formulation of the new mustard oil as 3-methyl-3-butenyl isothiocyanate (II).

On treatment with ammonia, (II) was converted into the crystalline 1-(3-methyl-3-butenyl)-thiourea, $C_6H_{12}N_2S$, m.p. 80°, exhibiting the expected bands in the infra-red spectrum.

Adopting the semi-systematic nomenclature proposed by Ettlinger and Dateo,[4] the glucosidic anion occurring in *Capparis linearis* Jacq. is: *3-methyl-3-butenylglucosinolate*. Although the isopentenyl structure of the derived isothiocyanate might superficially suggest an isoprenoid origin it seems more likely, on basis of the current knowledge of the biosynthesis of isothiocyanate-producing glucosides,[5] that homologization of intermediates on the valine-leucine metabolic pathways is involved in the *in vivo* synthesis of the thioglucoside (I).

* Part LIII of a series of papers on isothiocyanates. Part LII: *Acta Chem. Scand.* 17 (1963) 2562.

** The composition indicates that the new glucoside ion is isomeric with that of glucobrassicanapin, previously identified as a constituent of rape seed.[2]

Experimental. Paper chromatography. A 70 % methanolic extract of dry leaves of *Capparis linearis* Jacq. was chromatographed on paper (Schleicher and Schüll 2043b) in butanol:ethanol:water (4:1:4). On spraying with silver nitrate one glucoside spot was observed possessing an R_B-value * of 1.05. Enzymic hydrolysis of the extract, freed of methanol, produced an ether-soluble isothiocyanate which was converted into the corresponding thiourea on reaction with methanolic ammonia. On paper chromatography in water-saturated chloroform the thiourea migrated at a rate corresponding to an R_{Ph}-value of 0.95.[7] The presence of sulphate ions in the aqueous phase was demonstrated by precipitation as $BaSO_4$, and glucose was identified by paper chromatography.

Isolation of glucoside. Dry leaves (500 g) of *C. linearis* were disintegrated in 70 % methanol (3 l) in a Waring-Blendor and the suspension was refluxed for 15 min. The procedure was repeated with two fresh portions (each 2 l) of the same solvent. After filtration on Celite, the combined extracts were concentrated at 60° to a volume of 1.5 l. This solution was then applied to an anion exchange resin column (Amberlite IR-4B,H^+-form, 3 × 85 cm) which was subsequently washed with water. The glucoside was eluted by passing a 1 % K_2SO_4-solution through the column. The glucoside-containing fractions were combined and evaporated to dryness. Repeated extractions with anhydrous methanol served to remove the glucoside from inorganic material. After concentration to dryness, a total of 8 g of amorphous glucoside was obtained, yet still contaminated with some inorganic salt.

Tetramethylammonium salt of glucoside tetraacetate. An aliquot (300 mg) of the above glucoside was suspended in anhydrous pyridine (5 ml) and acetic anhydride (5 ml) was added. After two days at room temperature the dark-brown suspension was evaporated to dryness and triturated with anhydrous ether. The solid (120 mg) was dissolved in a few drops of methanol and applied to a small column of neutral alumina. Elution with ethyl acetate, containing increasing amounts of methanol, yielded a total of 60 mg of amorphous glucoside acetate, which was dissolved in water and passed through an ion exchange column (Amberlite IR-120), loaded with $(CH_3)_4N^+$-ions. The eluate was concentrated to dryness, and the solid was recrystallized twice from ethanol, containing a little ether, to give the colourless tetramethylammonium glucoside tetraacetate hemihydrate (16 mg), m.p. 168–171° (decomp.) $[\alpha]_D^{24}$ −22° (*c* 0.3, H_2O). (Found: C 45.09; H 6.36; N 3.95. Calc. for $C_{24}H_{40}O_{13}N_2S_2$, $0.5H_2O$: C 45.21; H 6.48; N 4.39).

An aliquot of the crystalline tetraacetate was heated at 60° for 2 h with 20 % HCl. Paper chromatography served to establish the presence of hydroxylamine in the hydrolysed solution.

Isolation of isothiocyanate. The amorphous glucoside (1 g) was dissolved in citrate buffer (15 ml, pH 6.5). A crude myrosinase solution (3 ml) and a trace of ascorbic acid were added, and the enzymic hydrolysis was allowed to proceed for 2 h at room temperature, when the isothiocyanate was removed by steam distillation and isolated from the distillate by ether extraction. After further purification by preparative gas chromatography a homogeneous specimen of 3-methyl-3-butenyl isothiocyanate was obtained as a colourless liquid (30 mg), n_D^{22} 1.519. (Found: C 56.92; H 7.20; N 10.83. Calc. for C_6H_9NS: C 56.65; H 7.13; N 11.01). Conspicuous IR-bands appeared at 900, 1340, 1365, 1450, 1645, 2090, 2180, 2900, and 3030 cm^{-1} (neat liquid). Mass- and NMR-spectra were likewise determined on this specimen.

1-(3-Methyl-3-butenyl)-thiourea. The isothiocyanate liberated as above from another glucoside aliquot (600 mg) was treated with methanolic ammonia to give a partly crystalline thiourea (29 mg) which separated from benzene:pentane as colourless, flat prisms (10 mg), m.p. 80°. (Found: C 49.66; H 8.48; N 19.29. Calc. for $C_6H_{12}N_2S$: C 49.98; H 8.39; N 19.43).

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* *i.e.* the R_F-value relative to that of glucotropaeolin.[6]

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Polarographic Investigations on Formylguaiacols and Veratrols

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The reactivity of guaiacols and veratrols is a problem of both theoretical and practical interest which has been extensively studied. In the present investigation the reactivity of various sites in the aromatic nucleus in these compounds has been studied using polarographic half-wave potentials of formylderivatives.

Experimental. The polarographic experiments using a dropping mercury electrode were performed in the usual manner[1] at 25 ± 0.1°C with a Cambridge General Purpose polarograph equipped with an electronic recorder. The exact potentials were read from a Radiometer PHM 4 pH-meter and the resistances of the solutions from a Philips PR 9500/01 conductometer. The height of the mercury column was 58.5 cm, the drop weight 2.537 $mg.s^{-1}$ and the dropping time in 0.1 N KCl 3.50 s. A saturated calomel electrode (SCE) was used as a reference electrode and the measurements were made in 0.1 N NH_4Cl −40 % ethanol-water mixtures. No maximum suppressor was used. The accuracy obtained was ± 0.01 V.

The aldehydes investigated were of commercial origin. They were purified before use by sublimation *in vacuo*, distillation and recrystallisation. Ethanol of spectrograde quality (AaS) from Oy Alkoholiliike Ab, Helsinki, showed no wave in the region investigated and was used as such.

Results. The results of the polarographic measurements are collected in Table 1.

Preliminary investigations on the dependence of halfwave potential and current on pH, concentration, and solvent, and the slope of the polarographic wave indicated that the present compounds follow generally accepted patterns for the reduction of aromatic monosubstituted aldehydes, *cf. e.g.* Ref. 2.

It has previously been found for guaiacylic as well as for other polysubstituted aromatic compounds in connection with

Table 1. Polarographic half-wave potentials (SCE) $E_{½}$ of aromatic aldehydes at 25°C in 0.1 N NH_4Cl−40 % ethanol-water mixtures. Concentration of aldehyde: 0.001 M.

No.	Substance		$-E_{½}$ V	$\Delta E_{½}$ V
1	Benzaldehyde		1.32	reference
2	Formylphenol	*o*-	1.38	−0.06
3		*m*-	1.31	+0.01
4		*p*-	1.47	−0.15
5	Formylanisol	*o*-	1.27	+0.05
6		*p*-	1.41	−0.09
7	Formylguaiacol	6-, (*o*-vanillin)	1.31	+0.01
8		5-, (iso- »)	1.40	−0.08
9		4-, (vanillin)	1.41	−0.09
10	Formylveratrol	6-	1.23	+0.09
11		4-	1.38	−0.06