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4,5,6,7-TETRAHYDROXYDECYL ISOTHIOCYANATE DERIVED FROM A GLUCOSINOLATE IN *CAPPARIS GRANDIS*

K. N. GAIND, K. S. GANDHI and T. R. JUNEJA

Department of Pharmaceutical Sciences, Panjab University Chandigarh - 160014, India

and

A. KJÆR and B. JUHL NIELSEN

Department of Organic Chemistry, The Technical University of Denmark, Lyngby, Denmark

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Abstract—Root material of *Capparis grandis*, of Indian origin, contains a free isothiocyanate, identified as 4,5,6,7-tetrahydroxydecyl isothiocyanate **1** by spectroscopical and chemical means, yet without specification as to its stereochemistry. The presence of the parent glucosinolate, and three other, unidentified glucosinolates in *C. grandis* has been noted. The distribution of glucosinolates within the genus *Capparis* is reviewed and the biosynthetic origin of the individual compounds discussed.

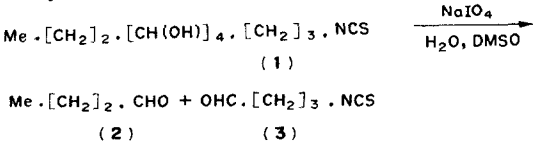
INTRODUCTION

In a search for biologically active compounds within the genus *Capparis* (Capparaceae), the species *C. grandis* L.f., commonly known in India as "Dhuti" or "Puchaonda", has been subjected to chemical examination. From dried root material an *iso*thiocyanate was isolated, the structure of which differed appreciably from those previously encountered as natural derivatives [1]. We report the results of our studies.

RESULTS

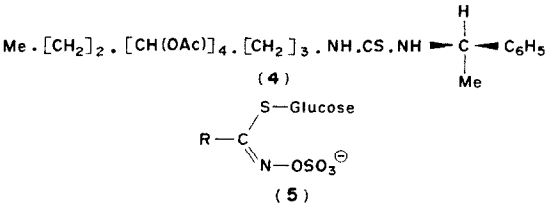
By ether extraction, dried, powdered roots of *C. grandis* afforded, in 0·7% yield, a dextrorotatory, crystalline compound **1**, $C_{11}H_{21}O_4NS$, possessing spectroscopic (UV and IR) and chemical properties in agreement with those expected for a tetrahydroxydecyl isothiocyanate. Thus, acetylation of **1** readily afforded a crystalline tetraacetate, $C_{10}H_{17}(OAc)_4NCS$, whereas reaction with ammonia yielded a thiourea, $C_{10}H_{17}(OH)_4NH.CS.NH_2$. The ^{1}H-NMR spectra of the tetraacetate and thiourea unequivocally revealed the presence in **1** of the following molecular fragments: $Me.CH_2.C$; $CH_2.CH_2.NCS$; two additional $C.CH_2.C$ groupings; four protons attached to hydroxy-substituted carbon atoms; and four hydroxylic protons. The UV spectrum of **1** in dioxane was unsusceptible to the addition of triethylamine, indicative of the absence of hydroxy-substitution at the 3-position of the carbon chain of **1**. Substitution at this position would have given rise, under these conditions, to a high-extinction band ($\log \epsilon \sim 4$) at about 250 nm, deriving from a 6-membered thionocarbamate formed upon intramolecular cyclization [2]. This observation, combined with the noted facile tetraacetylation of **1**, rendering the presence of tertiary hydroxy groups unlikely, permitted expansion of the structure formulation for **1** to: $Me.CH_2\ [(CH_2)_2(CHOH)_4](CH_2)_3.NCS$. Periodate oxidation provided the clue to the substitution pattern, furnishing two volatile carbonyl compounds, which could be trapped, separated, and identified as 2,4-dinitrophenylhydrazones. On MS and comparison with an authentic specimen the more lipophilic of the two was identified as the dinitrophenylhydrazone of butyraldehyde **2**, whereas the second derivative, in the mass

spectrometer, gave rise to a molecular ion at *m/e* 309 with the composition $C_{11}H_{11}O_4N_5S$ as determined by precise mass measurement. obviously the dinitrophenylhydrazone of 4-isothiocyanatobutyraldehyde **3**.



The combined evidence thus points to **1** being one of the 16 stereoisomeric 4,5,6,7-tetrahydroxydecyl *iso*thiocyanates.

Upon reaction of the acetate of **1** with (*R*)-1-phenylethylamine, the thiourea **4** was prepared, with a view to establishing its relative configuration, and hence the absolute configuration of **1**, by X-ray analysis. This work is now in progress.



Isothiocyanates encountered in higher plants, such as **1**, invariably derive from glucosinolates **5** by enzymic hydrolysis [3]. When a 70% MeOH extract of root material of *C. grandis* was subjected to PC analysis, the presence of four compounds, all susceptible to enzymic hydrolysis and behaving like glucosinolates, was revealed. The most lipophilic of these afforded **1** upon enzymic hydrolysis (see Experimental); the other, and minor glucosinolates remain unidentified. It seems likely that the substantial quantity of the non-volatile isothiocyanate **1**, extracted directly from the powdered roots, had been liberated from its glucosidic progenitor by unintentional enzymic hydrolysis during or after the collection and disintegration of the root material.

The isothiocyanate **1** has been subjected to biological studies. The results will appear elsewhere.

DISCUSSION

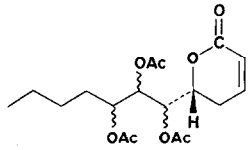
The tetrahydroxydecylglucosinolate (**5**, R = $Me.[CH_2]_2.[CH(OH)]_4.[CH_2]_3$), whence (**1**) derives, constitutes an interesting addition to the class of glucosinolates encountered in higher plants [1]. Within the family Capparaceae the pantropical genus *Capparis*, comprising more than 300 species, constitutes the largest and most important group. As far as we know, all *Capparis* species are sources of glucosinolates, and hence of *iso*thiocyanates, with the structurally simplest representative, methylglucosinolate **5** (R = Me), as the apparently most widely distributed compound. Substituted methylglucosinolates, however, are not rare within the genus. Table 1 presents a sum-

Table 1. Glucosinolates of the genus *Capparis*

Side-chain (R, in **5**)	Source (*Capparis* species)	References
Me	Numerous species	4, 5, 6, 7, 14
Pr ; Bu	*C. cartilagenea*; *C. galeata* Fresen.; *C. mitchellii* Lindbl.	4
CH_2:C(Me).$[CH_2]_2$	*C. linearis* Jacq.	9
Et.C(Me)(OH).CH_2	*C. spinosa* L.	10, 6
	C. ovata	6
MeS(O).$[CH_2]_3$	*C. spinosa* var., *C. ovata*	6
CH_2:CH.CH_2	*C. spinosa* var., *C. ovata*	6
Bu ; Me.(CHOH).$[CH_2]_2$; HO.$[CH_2]_4$; CH_2:CH.CH(OH).CH_2	*C. flexuosa* L. (Jamaica)	8
Et.CO.$[CH_2]_4$	*C. salicifolia* Griseb.	13
	C. flexuosa L. (Jamaica)	14
Pr.CO.$[CH_2]_3$	*C. angulata* Ruiz et Pav.	11
	C. spinosa var.; *C. ovata*	6
Pr.CO.$[CH_2]_4$	*C. salicifolia* Griseb.	12
$C_6H_5.CH_2$	*C. flexuosa* L. (Colombia)	5
3-Indolyl.CH_2	*C. ovata*	6
1-MeO-3-indolyl.CH_2	*C. spinosa* var. *deserti* Zoh.	6

mary of their chemical structures and botanical sources.

In the light of the generally accepted *in vivo* derivation of glucosinolates from α-amino acids [3,15], the elaboration of most side-chains listed in Table 1 from certain protein amino acids (Ala, Val, Leu, Met, Phe, Try), or their homologized counterparts, appears reasonably straightforward. Notable exceptions exist, however. Thus, it is not clear which amino acids the oxo-containing C-7 and C-8 chains, nor the here reported tetrahydroxylated C-10 array, are derived from. Experimental clarification of this point would be welcomed.



(6)

Me . $[CH_2]_n$. $[CH(OH)]_3$.CH_2OH

(7)

Long-chain, linear, vicinal tetrols are rare in Nature. Boronolide **6**, a constituent of *Tetradenia fruticosa* Benth. (Labiatae) [16], and the tetrols **7** (n = 13, 14 and 15), obtained from gum-resin of *Commiphora mukul* Engl. (Burseraceae) [17], are examples of such structures, yet only remotely related to that of **1**. The biosynthetic origin and detailed stereochemistry of **6** and **7** remain to be established.

EXPERIMENTAL

The root material of *Capparis. grandis*, used for the present work, was obtained by courtesy of The Botanical Survey of India, Western Circle Poona, where a sample specimen is kept under the file no. 104607. Microanalyses were performed by Mr. G. Cornali and his staff. M.ps were determined in capillary tubes in a heated bath and are uncorrected.

Isolation and properties of the isothiocyanate **1**. Dry, powdered root material of *C. grandis* (170 g) was extracted with Et_2O in a Soxhlet. Evaporation of the solvent gave a semi-solid residue (1·2 g) which was recrystallized from MeOH to give colourless needles (1·1 g), m.p. 178–180° (dec.), $[\alpha]_D^{24}$ + 6·4° (*c* 1, MeOH). An analytical specimen was produced by an additional recrystallization from MeOH, m.p. 178–179° (Found: C, 50·1; H, 8·0; N, 5·3; S, 12·1. $C_{11}H_{21}O_4NS$ requires: C, 50·2; H, 8·0; N, 5·3; S, 12·2). UV (in dioxane): λ_{max}: 246 nm (ε 1100), unchanged, over 3 days, after the addition of a few drops of Et_3N. IR (in KBr): strong bands at 2098 and 2180 cm^{-1} (NCS); and at 3250 cm^{-1} (OH). ^1H NMR-spectrum (in DMSO-d_6): δ 0·82 [distorted *t*; 3H; *J ca* 6 Hz; C<u>H</u>$_3$.CH_2]; δ 1·35–1·90 [*m*, 8H, 4 C<u>H</u>$_2$–]; δ 3·43 [narrow *m*; 2C<u>H</u>(OH); 2 O<u>H</u> (displaced to δ 3·98 on addition of D_2O)]; δ 3·62 [*t*; 2H; *J ca* 4Hz; –CH_2.C<u>H</u>$_2$.NCS]; δ 3·81 [*m*; 2H; 2C<u>H</u>(OH)]; δ 4·05–4·35 [*m*; 2H; 2O<u>H</u> (exchangeable on addition of D_2O)]; irradiation at δ 1·80 causes collapse of the δ 3·62 signal to a singlet.

O-Tetraacetate of the isothiocyanate **1**. The crystalline isothiocyanate **1** (25 mg) was acetylated with Ac_2O (2 ml) in pyridine (2 ml) at 20°. After 14 hr, the mixture was worked up to give the chromatographically homogeneous *O*-tetraacetate (29 mg) which, before analysis, was recrystallized from $CHCl_3$–hexane, m.p. 140–141° (Found: C, 52·8; H, 6·6; N 3·2. $C_{19}H_{29}O_8NS$ requires: C, 52·9; H, 6·8; N, 3·2). MS (70 eV; inlet 70°): $M^{\oplus}$ at *m/e* 431 (calc. 431); base peak: *m/e* 371 (M-60). IR (in KBr): 1740 (vs) (ester CO); 2105 (vs) and 2180 (vs) (NCS); 1218 (vs) and 1235 (vs) cm^{-1} (ester C–O– stretching); no OH-absorption was observed. ^1H NMR-spectrum (in $CDCl_3$): δ 0·9 [C<u>H</u>$_3$.CH_2], δ 1·2–1·8 [4 C<u>H</u>$_2$–]; δ 2·07, 2·08, 2·12, and 2·16 [4 OAc]; δ 3·52 [–CH_2.C<u>H</u>$_2$.NCS]; δ 4·9–5·2 [2 C<u>H</u>(OAc)]; δ 5·25 [2 C<u>H</u>(OAc)].

Thiourea of the isothiocyanate **1**. When the isothiocyanate **1** was kept in MeOH, satd at 0° with NH_3, for 4 hr, a quantitative yield was obtained of the corresponding thiourea, m.p. 187°. An analytical specimen was produced by recrystallization from H_2O, m.p. 188–189°; $[\alpha]_D^{28}$ + 1·9° (*c* 0·5, MeOH) (Found: C, 47·0; H, 8·6; N, 9·8. $C_{11}H_{24}O_4N_2S$ requires: C, 47·1; H, 8·6; N 10·0). UV (in EtOH): λ 241 nm (ε 9700). TLC Sil gel, EtOAc–MeOH (9:1): R_f 0·18.

Periodate fission of the isothiocyanate **1**. A soln of **1** (7·3 mg) in a mixture of H_2O (8 ml) and DMSO (2·5 ml), to which $NaIO_4$ (110 mg) had been added, was stirred at room temp. for 1 hr and then distilled. The volatiles were absorbed in 2,4-dinitrophenylhydrazine reagent solution, and the resulting ppt. chromatographed on Si gel [heptane–Et_2O (5:1)] giving two yellow bands. These were separately eluted with Et_2O, and the residues recrystallized from H_2O–EtOH before MS. The fastest moving dinitrophenylhydrazone exhibited a MS identical with that of an authentic specimen of the dinitrophenylhydrazone of butyraldehyde, $M^{\oplus}$ at *m/e* 252. The slower moving, yellow derivative exhibited a MS with the highest peak at *m/e* 309 and a fragmentation pattern characteristic for 2,4-dinitrophenylhydrazones [18,19]. Precise mass measurement of the molecular ion gave: 309·0534; $C_{11}H_{11}O_4N_5S$ requires: 309·0532, confirming the identity of the parent aldehyde as 4-*iso*thiocyanatobutyraldehyde **3**.

Thiourea **4**, *produced from the tetra-O-acetate of* **1** *and* (R)-1-*phenylethylamine*. A soln of the tetra-*O*-acetate of **1** (51 mg) and (*R*)-(+)-1-phenylethylamine (60 mg) in Et_2O (10 ml) was evaporated to dryness after 1 hr at room temp. The residue was purified by chromatography on Si gel plates (Et_2O), yielding the expected, crystalline thiourea **4** (60 mg) which was recrystallized from EtOH–H_2O to give an analytical specimen, m.p. 182–183°; $[\alpha]_D^{20}$ – 11·2° (*c* 1, $CHCl_3$) (Found: C, 58·6; H, 7·4; N, 5·1. $C_{27}H_{40}O_8N_2S$ requires: C, 58·7; H, 7·3; N, 5·1).

Glucosinolates in C. grandis. Powdered root material of *C. grandis* (50 g) was extracted with 70% MeOH. The syrupy residue was separated, on preparative PC [Whatman paper No. 3M; BuOH–EtOH–H_2O (4:1:4); spray reagent Ag^+/NH_3], into 4 glucosinolates, with the R_f-values: 0·15; 0·29; 0·39, and 0·92, the latter representing the major constituent. Each component underwent the reactions characteristic for glucosinolates: susceptibility to hydrolysis with myrosinase, release of hydroxylamine and glucose on acid hydrolysis, and characteristic coloration with Ag^+ [3], but only the glucosinolate with R_f 0·92 was subjected to a more detailed study. On enzymic hydrolysis, this glucosinolate afforded the *iso*thiocyanate **1**, which was converted into the corresponding thiourea on reaction with ammonia, having the R_f-value 0·18 when chromatographed alone or in admixture with the thiourea derived from **1**, in the solvent system stated above. Comparitive chromatography in other solvent systems further served to establish the identity of the two thiourea preparations.

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