Phytochemistry, 1971, Vol. 10, pp. 3155 to 3160. Pergamon Press. Printed in England.

GLUCOSINOLATES IN *CAPPARIS FLEXUOSA* OF JAMAICAN ORIGIN

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(*Received* 10 *March* 1971)

Abstract—The glucosinolates in dried leaves of *Capparis flexuosa* (L.)L., collected in Jamaica, were examined by identifying the derivatives of the isothiocyanates initially produced by their enzymic hydrolysis. Evidence is presented for the existence in the leaves of five C_4 glucosinolates, *viz.* butyl, 3-hydroxybutyl-, 4-hydroxybutyl-, 3-butenyl-, and 2-hydroxy-3-butenylglucosinolate, the first three of which represent new natural products. The results are at variance with those obtained elsewhere on the same species from the same locality. The reasons for the discrepancy are discussed. The results also deviate from those obtained in this laboratory in a previous study of allegedly the same species, collected in Colombia. Again, the difference is commented upon.

INTRODUCTION

THE PLANT *Capparis flexuosa* (L.)L. (Capparidaceae), of Jamaican origin, was given its present name by Linnaeus in 1762 (see Ref. 1). Its geographical range is generally stated as Southern Florida, the Antilles, the semi-arid portions of Venezuela, Colombia, and Western Ecuador; both coasts of Central America to Southern Mexico, Paraguay, Brazil (Santa Catarina) and, perhaps, Peru.

A few years ago, benzylglucosinolate was established in this laboratory[2] as the sole glucosinolate present in detectable amounts in dried leaves of *C. flexuosa* collected in Colombia. This finding was at variance with the reported presence of methyl- and 4-oxoheptylglucosinolate, both previously encountered in other members of the genus *Capparis*,[3] in dried leaves of *C. flexuosa* of Jamaican provenance.[4,5] The observed discrepancy aroused our interest in a comparative study of materials of Jamaican origin. In the present communication we report and discuss the interesting and unexpected results.

RESULTS

The leaf material employed in the present study was collected in the spring of 1970 on Mona Road, St. Andrew, Jamaica. After drying, it was generously placed at our disposal by Dr. K. L. Stuart, Chemistry Department, University of the West Indies, Kingston, Jamaica.*

On paper chromatography of a methanolic leaf extract, three glucosinolate spots were observable. When a glucosinolate solution, produced from 500 g of dried leaves by methanol

* The collection was identified as *Capparis flexuosa* (L.) L. by Dr. Dennis Adams, Botany Department, University of the West Indies. Mona, Kingston 7, Jamaica (K. L. Stuart, personal communication).

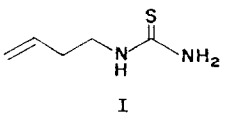
[1] W. FAWCETT and A. B. RENDLE, *Flora of Jamaica* III, 234 (1914).
[2] R. GMELIN and A. KJÆR, *Phytochem.* **9** ,601 (1970).
[3] M. G. ETTLINGER and A. KJÆR, in *Recent Advances in Phytochemistry* (edited by T. J. MABRY, R. E. ALSTON and V. C. RUNECKLES), Vol. 1, p. 58, Appleton–Century–Crofts, New York (1968).
[4] I. V. BROWN and K. L. STUART, *Phytochem.* **7**, 1409 (1968).
[5] I. V. BROWN, *Glucosinolates and Other Natural Products from Jamaican Plants.* M.Sc. Thesis, University of the West Indies, p. 65 (1969).

extraction, followed by ion exchange purification, was subjected to enzymic hydrolysis by a crude myrosinase preparation, a mixture of chloroform-soluble products resulted. Partial separation of these was achieved by chromatography on silica gel, whereby three fractions were obtained, marked (i), (ii), and (iii) according to the order in which they emerged from the column.

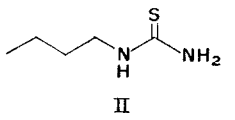
The oily fraction (i), consisting, according to spectroscopy, of one or more isothiocyanates, was converted into a solid thiourea fraction (90 mg) on treatment with methanolic ammonia. Paper chromatography in two solvent systems showed it was a mixture of two thioureas in approximately equal amounts. Countercurrent distribution of the mixture (60 plates) between chloroform and water resulted in partial separation, and homogeneous specimens of the two thioureas were obtained from the appropriate tubes. One (34 mg), proved identical with a previously prepared specimen of 1-(3-butenyl)-thiourea[6,7] (I), whereas the second thiourea (23 mg), on critical comparison with a synthetic specimen, was found identical with 1-butyl-thiourea (II), obviously deriving from butyl isothiocyanate. A previous claim[7a] as to the presence of the latter isothiocyanate in disintegrated, fresh cabbage has not been substantiated by subsequent work.

The oily fraction (ii) (82 mg), consisting of isothiocyanates and other components according to spectroscopic analyses (UV, IR, NMR and MS), was also treated with ammonia in methanol, and the resulting mixture was subjected to chromatography on silica gel (EtOAc, with increasing amounts of EtOH). Four fractions, (a–d), each containing essentially one component according to TLC, were obtained and separately subjected to further studies.

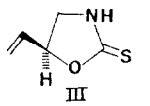
(a) The most lipophilic and largest fraction (55 mg) was purified by re-crystallization



I



II



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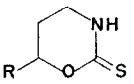
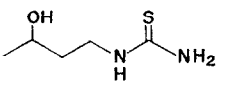
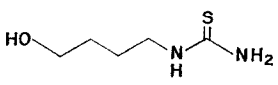


IV

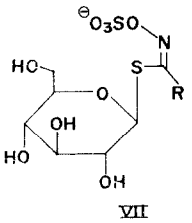
a : R = CH_3
b : R = $CH_3SCH_2CH_2$
c : R = $CH_3SO_2CH_2CH_2$



V



VI



VII

a, R: $CH_3CH_2CH_2CH_2$
b, R: $CH_3CHOHCH_2CH_2$ (config. unknown)
c, R: $HOCH_2CH_2CH_2CH_2$
d, R CH_2=$CHCH_2CH_2$
e, R CH_2=CH-$CH(OH)CH_2$,(*S*)-config.

[6] A. KJÆR, K. RUBINSTEIN and K. A. JENSEN, *Acta Chem. Scand.* **7**, 518 (1953).
[7] M. G. ETTLINGER and J. E. HODGKINS, *J. Am. Chem. Soc.* **77**, 1831 (1955).
[7a] S. D. BAILEY, M. L. BAZINET, J. L. DRISCOLL and A. I. MCCARTHY, *J. Food. Sci.* **26**, 163 (1961).

from water to give 19 mg of the *levo*rotatory (*S*)5-vinyloxazolidine-2-thione (III), identified upon comparison with an authentic specimen isolated by enzymic hydrolysis of (*R*)2-hydroxy-3-butenylglucosinolate, a long established constituent of, e.g. seed of rape (*Brassica napus* L.).[8–11]

(b) The subsequent, crystalline fraction (17 mg) was *dextro*rotatory, and possessed a UV-spectrum (λ_{max}^{EtOH} 252 nm) characteristic of the 6-membered tetrahydro-1,3-oxazine-2-thione system previously encountered in glucosinolate studies.[12] In fact, after purification, the isolated compound (m.p. 136–137°) possessed IR, UV and mass spectra indistinguishable from those of an authentic, synthetic specimen of (±)-6-methyl-tetrahydro-1,3-oxazine-2-thione (m.p. 142°) (IVa).[12] The 6-methyl-substituted ring compound has not previously been reported in plants. Its absolute configuration, almost certainly the same as that of the 3-hydroxybutylglucosinolate whence it most likely derives, is still unknown. It is of interest, however, that the chirality of (+) (IVa), according to CD-studies, appears to be opposite to that of the *levo*rotatory compounds (IVb) and (IVc), both previously reported enzymic hydrolysis products of glucosinolates in *Erysimum virgatum* Roth.[13]*

(c) The third fraction consisted of oily material (9 mg) which could be purified only with difficulty to give a minute quantity, insufficient for determination of its optical rotation, of a semi-solid compound, homogeneous on TLC and possessing IR and mass spectra almost identical with those of a synthetic specimen of (±)-1-(3-hydroxybutyl)-thiourea (V).[12]†

(d) The most hydrophilic, oily fraction (10 mg) was converted into a crystalline thiourea which, on critical comparison, proved identical with a synthetic specimen of the previously unknown 1-(4-hydroxybutyl)-thiourea (VI) (see Experimental).

The crystalline fraction (iii) (6 mg) was found to consist of essentially homogeneous 6-methyl-tetrahydro-1,3-oxazine-2-thione (IVa), identical with the specimen obtained from fraction (ii) as described above.

DISCUSSION

In view of the above established identity of the various derivatives isolated subsequent to enzymic hydrolysis of the glucosinolates in leaves of the Jamaican *C. flexuosa*, and considering the established lack of variation in the general structure (VII) of naturally occurring glucosinolates (see e.g. Ref. 3), it appears reasonable to conclude that all of the detectable glucosinolates in the leaf material studied are of the usual type and contain an unbranched C_4-side chain, saturated or terminally unsaturated, with or without hydroxy-substitution, as summarized in structures (VII, a–e).

On enzymic hydrolysis, the corresponding isothiocyanates, R-NCS, can be envisaged as the primarily formed products, besides glucose and sulphate. The β-hydroxy-substituted species, derived from (VIIe), undergoes spontaneous cyclization to the oxazolidinethione (III), whereas the other isothiocyanates, on treatment with ammonia, afford either thioureas

* Referred to previously as *E. hieracifolium* L.[13] Expert studies have indicated that the species employed in our studies was, in fact, *E. virgatum* Roth of the *E. hieracifolium* group (presumably identical with *E. durum* J. et C. Presl.) (Prof. C. Favarger, Neuchatel and Dr. A. Polatschek, Vienna; private communications).

† On repetition of the previously reported synthesis of the racemic thiourea, correctly analysing preparations with m.p.85° (not 115° as reported[12]) were invariably obtained (cf. Experimental).

[8] E. B. Astwood, M. A. Greer and M. G. Ettlinger, *J. Biol. Chem.* **181**, 121 (1949).
[9] M. G. Ettlinger, *J. Am. Chem. Soc.* **72**, 4792 (1950).
[10] A. Kjær, B. W. Christensen and S. E. Hansen, *Acta Chem. Scand.* **13**, 144 (1959).
[11] M. E. Daxenbichler, C. H. VanEtten and I. A. Wolff, *Biochem.* **4**, 318 (1965).
[12] A. Kjær and R. B. Jensen, *Acta Chem. Scand.* **12**, 1746 (1958).
[13] A. Kjær and A. Schuster, *Acta Chem. Scand.* **24**, 1631 (1970).

(I, II, V and VI), or, competitively, in the case of γ-hydroxy-substitution, a tetrahydro-1,3-oxazine-2-thione (IVa) (see Ref. 12). Thus, the above described reaction products can be satisfactorily accounted for in terms of the glucosinolates (VII, a–e). Of these, the unsaturated species (VIId, VIIe) have been repeatedly encountered in various crucifers (*Brassica* species and others),[3,14] whereas the saturated C-4 glucosinolates, with or without hydroxy-substitution, (VIIa, VIIb, VIIc), represent novelties within this class of natural products. From comparison and experience, it seems reasonable to assume that the glucosinolates with R_B-values (in solvent system A) of 0·86, 0·65, and 0·38 (cf. Experimental) represent (VIIa), (VIId), and the sum of (VIIb), (VIIc), and (VIIe), respectively.

Much progress has been made within recent years in our understanding of the general biogenetic pathway from α-amino acids to glucosinolates (see e.g. Ref. 3). However, attempts to account specifically for the steps, most likely parallel or partly coinciding, leading to the glucosinolates (VIIa)–(VIIe) will remain pure speculation until experiments are on hand.

No obvious explanation can be offered for the discrepancy between the above results and the previously reported finding of methyl- as the major, and, less convincingly, 4-oxo-heptyl-glucosinolate as the sole minor constituent in dried leaves of *C. flexuosa* collected on the same locality.[4,5] In the present studies, not even trace amounts of methylglucosinolate were observed on chromatographic examination of concentrated extracts. To what extent age or growth phase on one hand, and season, drying procedure, and other environmental factors on the other hand, may influence the glucosinolate pattern is unknown. It would appear of interest to obtain a better understanding of the very marked and unusual variations observed in the present case.

Again, the discrepancy between the above results and the previously reported finding of benzylglucosinolate in allegedly the same species of Colombian origin,[2] may reflect still another variation conditioned by external factors. In this case, however, it might be valuable to know if the similarly named taxa in Jamaica and on the South American continent are, in fact, the same plants.

EXPERIMENTAL

The plant material used in the present study was collected in April 1970 on Mona Road, St. Andrew, Jamaica by Dr. L. K. Stuart, and dried in an IR drying oven immediately after collection. The dried leaves (1 kg) were sent by air to the Danish laboratory and extracted shortly after arrival.

Paper chromatograms of glucosinolates were run in the solvent systems (A), BuOH–EtOH–H_2O (4:1:4), and (B), BuOH–pyridine–H_2O (6:4:3), as recently described.[15] Column chromatography was performed on silica gel Merck (0·05–0·2 mm). TLC plates were sprayed with $AgNO_3$, or, in the case of thioureas, with Grote's reagent. M.ps are uncorrected and determined in an electrically heated bath. IR spectra were recorded in KBr pellets, mass spectra with a Perkin–Elmer 270 apparatus (ionizing potential 70 eV), and CD-curves with a Roussel–Jouan Dicrographe.

Chromatography and Enzymic Hydrolysis of Glucosinolates

On paper chromatography of a 70% MeOH extract of ground, dry leaves of *C. flexuosa* (L.) L., three spots were observed in solvent system (A), with R_B-values[15] of 0·38, 0·65, and 0·86, in system (B), only two spots appeared, R_B 0·72, and 0·97.

The disintegrated, dry leaves (500 g) were extracted on refluxing with 70% MeOH (7 l.). After filtration and pressing of the filter cake, extraction was repeated with a fresh portion (3 l.) of 70% MeOH. The combined filtrates were concentrated *in vacuo* to about 1 l. and filtered through Hyflo Supercel. The filtrate was passed through a column of Dowex 1 × 1 ion exchange resin, on the Cl^--form; the column was rinsed with water (300 ml), and the glucosinolates were eluted by passing a 5% K_2SO_4 solution through the column,

[14] A. KJÆR, *Fortschr. Chem. Org. Naturstoffe* **18,** 122 (1960).
[15] R. GMELIN and A. KJÆR, *Phytochem.* **9,** 591 (1970).

200 ml fractions being collected. Paper chromatographic analysis served to establish the presence of the total glucosinolate content in fractions Nos. 2–9. These were combined and evaporated to dryness. The solid residue, containing large amounts of inorganic salt, was repeatedly extracted with hot 80% ethanol. The combined extracts were evacuated *in vacuo* until free of ethanol. A citrate buffer (pH 6·5) (300 ml) was added to the solution, together with a few ml of a cell-free myrosinase preparation,[16] and a trace of ascorbic acid. After standing for 5 hr at 37°, the enzymically hydrolysed mixture was extracted four times with $CHCl_3$, and the combined extracts (350 ml) were dried over Na_2SO_4.

Separation and Derivatizing of Hydrolysis Products

The $CHCl_3$-solution was poured onto a dry column of silica gel (50 g), and 25-ml fractions were collected. The fractionation was controlled by TLC chromatography (in $CHCl_3$–EtOH (95:5), or EtOAc). When the most lipophilic fraction, (i), had emerged (Nos. 1–7), more $CHCl_3$ (100 ml), followed by EtOAc (150 ml) were passed through the column to give fractions (ii) (Nos. 11–13), and (iii) (Nos. 14–16).

Fraction (1) was treated overnight with a few ml of methanol, saturated at 0° with NH_3. The residue (90 mg) contained two thioureas, as seen from paper chromatography in two solvent systems. Partial separation of these was achieved by countercurrent distribution between chloroform and water in an all-glass Craig-type apparatus with 60 plates (each 20 ml). After a complete cycle of 60 transfers (water as the moving phase), paper chromatographic analysis indicated that fractions Nos. 24–32 and 43–56 contained essentially homogeneous solutions of the two thioureas. The contents of the former set of tubes were combined and evaporated to dryness; the residue (23 mg) was recrystallized from benzene to give a nicely crystalline product, m.p. 71–72°, alone or in admixture with a synthetic preparation of 1-butylthiourea (II). Coinciding IR and mass spectra further served to establish their identity. The combined plates Nos. 43–56 were likewise concentrated to dryness, and the residue (34 mg) was recrystallized from H_2O to give colourless needles with m.p. 62°, undepressed on admixture with a synthetic specimen of 1-(3-butenyl)-thiourea (I);[6] again, identical IR and mass spectra confirmed the identity.

Fraction (ii) was treated with ammonia in methanol as described above to give a mixture (85 mg) according to paper and TLC chromatography. A column was prepared of H_2O-deactivated silica gel (30 g), suspended in EtOAc, and the mixture was chromatographed with EtOAc, from fraction 12 containing EtOH (2%), increased to 5, 10, 15, and 20% for each 50 ml; 20-ml fractions were collected.

Fraction (a), Nos. 3–5, consisted of oily material (55 mg); on cooling, a hot aqueous solution deposited colourless crystals (19 mg), m.p. 44°, undepressed on admixture with an authentic specimen of (*S*) 5-vinyl-oxazolidine-2-thione (III); the isolated compound had $[\alpha]_D^{24}$ −72° (c 1·4, $CHCl_3$) (reported value: −76·8° (*c* 2·0, $CHCl_3$)[11]), and exhibited UV, IR and MS indistinguishable from those of an authentic sample isolated from rape seed.

Fraction (b), Nos. 7–9, was crystalline (17 mg). On the basis of identical m.p. and IR spectra, it was combined with fraction (iii) (6 mg) (see below) and purified by chromatography on silica gel in EtOAc, followed by recrystallization from chloroform–hexane to give colourless needles (7 mg), m.p. 137°, $[\alpha]_D^{20}$ +59° (*c* 0·6, $CHCl_3$). The UV and mass spectra were indistinguishable from those of an authentic specimen (m.p. 142°) of racemic 6-methyl-tetrahydro-1,3-oxazine-2-thione (IVa);[12] surprisingly, even the solid phase IR spectra of the naturally derived *dextro*rotatory enantiomer and the racemate were superimposable. The CD-curve (in CH_3CN) showed extremes at: 287 ($\Delta\epsilon$, −1·7), 250 ($\Delta\epsilon$, +2·3), and 209 nm ($\Delta\epsilon$, +1·7). The corresponding values determined in CH_3CN for the 6-(2-methylthioethyl)- (IVb) and 6-(2-methylsulphonyl-ethyl)-derivatives (IVc)[13] were: 289 ($\Delta\epsilon$, +2·5), 251 ($\Delta\epsilon$, −3·7), 213 ($\Delta\epsilon$, −2·4), and 290 ($\Delta\epsilon$, +1·7), 250 ($\Delta\epsilon$, −2·3), 207 ($\Delta\epsilon$, −1·6) nm, respectively, indicating a chirality of (IVa) opposite to that of (IVb) and (IVc).

Fraction (c), Nos. 25–35, consisted of oily material (9 mg) which was subjected to renewed chromatography in EtOAc on silica gel to give 3·7 mg of a partly purified product which after repeated column chromatography was chromatographically homogenous (0·9 mg), but only partly crystalline. Due to lack of material, no optical rotation was determined. The mass spectrum suggested that the compound was 1-(3-hydroxybutyl)-thiourea (V), of unknown stereochemistry. Support for this hypothesis was sought by comparison with a synthetic specimen of the racemic modification. The latter was prepared from the racemic 3-hydroxybutyl isothiocyanate, essentially as described previously.[12] The product obtained after treatment of the isothiocyanate with ammonia was fractionated into (±)-6-methyl-tetrahydro-1,3-ozaxine-2-thione (IVa), formed by competitive, base-induced, intramolecular cyclization,[12] and (±)-1-(3-hydroxybutyl)-thiourea (V), by chromatography on silica gel in $CHCl_3$ with increasing amounts of EtOH. The thiourea was crystallized twice from EtOAc–hexane to give colourless needles, m.p. 85° (previously reported 115°[12]). (Found: C, 40·56; H, 8·03; N, 18·92. $C_5H_{12}ON_2S$ required: C, 40·53; H, 8·17; N, 18·91.) The authors did not observe m.ps higher than 85° during the present work. On comparison with the synthetic thiourea, the naturally derived thiourea showed an identical mass spectrum, and an IR spectrum virtually identical with that of the synthetic sample, the minor deviations probably reflecting the different stereochemistry of the two specimens.

[16] C. NEUBERG and J. WAGNER *Biochem. Z.* **174,** 457 (1926).

Fraction (d), Nos. 38–40, appeared as oily material (10 mg) which was dissolved in a minimum amount of hot EtOAc. On cooling, colourless crystals separated, m.p. 95–96°, which, according to IR and mass spectrometry, most likely consisted of the previously unknown 1-(4-hydroxybutyl)-thiourea (VI). On paper chromatography in solvent system (A) the thiourea gave one spot with an R_f-value of 0·64; the 3-hydroxy-isomer (V), run on the same chromatogram, appeared with an R_f-value of 0·69. In order to substantiate the identification, a synthetic specimen of 1-(4-hydroxybutyl)-thiourea was prepared in the following way: 4-aminobutanol[17] (2·0 g) and thiocarbonyl chloride (2·9 g) were added to a vigorously stirred system of $CHCl_3$ (45 ml) and 5% $NaHCO_3$ solution (100 ml), kept at 0–5°, for about 0·5 hr. The phases were separated, and the aqueous phase extracted with $CHCl_3$ (50 ml), which was added to the first $CHCl_3$-phase. After cooling in dry ice, liquid NH_3 (100 ml) was added to the $CHCl_3$ solution. After several days, a solid (polymer) was removed from the heated $CHCl_3$ solution which on evaporation gave a residue, recrystallized from CH_3NO_2 to give a brownish product (90 mg). The latter was purified by repeated recrystallizations from EtOAc to give colourless needles, m.p. 103–104°. (Found: C, 40·22; H, 8·14; N, 18 44.) A mixture of the naturally derived thiourea (95–96°) and the synthetic specimen had m.p. 95–96°, indicating that the former was not entirely homogeneous. On the other hand, the two specimens had identical R_fs, IR, UV, and mass spectra.

Fraction (*iii*) contained a crystalline product (6 mg) which, according to IR and mass spectra, consisted of 6-methyl-tetrahydro-1,3-oxazine-2-thione (IVa). It was therefore combined with the (b) portion of fraction (ii) (*vide supra*).

Acknowledgements—The authors are very grateful to Dr. K. L. Stuart, Chemistry Department, University of the West Indies, for his assistance in collecting the plant material employed in the present work, and to Dr. Dugand, Barranquilla, Colombia for his valuable botanical expert advice. Thanks are further due to Dr. P. Laur for help with the CD-measurements, to Dr. S. Refn for IR-spectra, and to Mr. S. Buhl for assistance in the synthetic work. Microanalyses were performed by Mr G Cornali and his staff.

[17] J. R. PIPER, C. R. STRINGFELLOW, JR. and T. P. JOHNSTON, *J. Heteroc. Chem.* **4**, 298 (1967).