ACTA CHEMICA SCANDINAVICA 24 (1970) 1631–1638

Glucosinolates in *Erysimum hieracifolium* L.; Three New, Naturally Occurring Glucosinolates

ANDERS KJÆR and ANGELICA SCHUSTER

Department of Organic Chemistry, Technical University of Denmark, DK-2800 Lyngby, Denmark

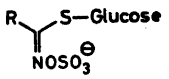
Seed extracts of *Erysimum hieracifolium* L. (Cruciferae) contain at least five glucosinolates. Upon enzymic hydrolysis, they produce a series of isothiocyanates which can be separated chromatographically.

By application of chemical and spectroscopic methods the isothiocyanates are identified as: (1) 5-methylthiopentyl, (2) (*R*)-5-methylsulphinylpentyl, (3) 3-hydroxy-5-methylthiopentyl, (4) 3-hydroxy-5-methylsulphinylpentyl, and (5) 3-hydroxy-5-methylsulphonylpentyl isothiocyanate. Of these, (3), (4), and (5) undergo facile spontaneous or base-induced cyclizations to the corresponding, chiral 6-substituted tetrahydro-1,3-oxazine-2-thiones.

Determination of the absolute configuration of the naturally derived isothiocyanates (3), (4), and (5) is a subject of present studies.

The occurrence of 5-methylthiopentylglucosinolate, and its higher oxidized analogues, is new to the genus *Erysimum*. The possible taxonomic significance hereof is briefly discussed.

The genus *Erysimum* (Cruciferae) is one of great taxonomic complexity, even when limited to taxa indigenous to Northern and Central Europe. For this and other reasons, we considered the genus inviting for phytochemical studies and have subjected seeds of a number of *Erysimum* species to a detailed analysis for their contents of glucosinolates (I). The results will appear in a separate communication.[1]



(I)

In the course of these studies, however, seed extracts of *E. hieracifolium* L.* attracted our special interest, due to their complex and unusual

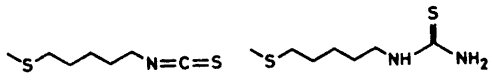
* The seed employed was produced by large scale cultivation in the Botanic Garden of the University of Copenhagen. Herbarium vouchers of the plants are deposited in the Botanic Museum of the University of Copenhagen.

glucosinolate patterns as revealed by routine paper-chromatographic analyses. Detailed investigations disclosed the presence in the extracts of a number of glucosinolates not previously encountered in Nature. We report the results here.

RESULTS

Paper chromatography, in two solvent systems (see Experimental), of a methanolic seed extract of *E. hieracifolium* revealed its contents of several glucosinolates. In order to obtain information about their chemical character, a purified extract of a larger seed sample (300 g) was subjected to enzymatic hydrolysis with a myrosinase preparation. The chloroform-soluble products formed in the enzymatic reaction (2.1 g) were fractionated by column chromatography on silica gel. Five main fractions, numbered 1 – 5 according to the order in which they appeared from the column, were collected and separately studied.

Fraction 1. Cautious evaporation of an aliquot of fraction 1 left an oil which by combined gas chromatography/mass spectrometry was identified as 5-methylthiopentyl isothiocyanate (II), a compound previously encountered as a product formed by enzymic hydrolysis of a glucosinolate in seeds of the crucifer *Berteroa incana* (L.) DC.,[2] but most likely present also in several



(II) (III)

species of the genera *Alyssum*[3] and *Lunaria*.[2] In order to substantiate the identification of (II), another aliquot was converted into the corresponding thiourea on reaction with ammonia. Critical comparison of the latter with an authentic specimen[2] proved it to be 1-(5-methylthiopentyl)-thiourea (III).

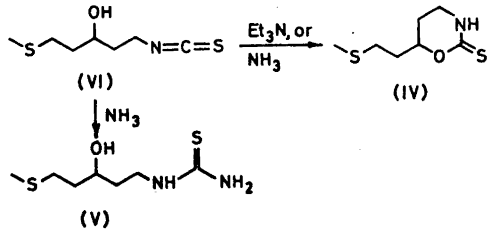
Fraction 2. This fraction was divided into two parts. (i) One was treated with methanolic ammonia, and the product mixture (300 mg) was separated by chromatography on silicagel. Two major products, A and B, were isolated in homogeneous form.

Compound A, m.p. 58 – 59°, was strongly levorotatory and possessed the composition $C_7H_{13}NOS_2$. This, together with its formation and spectroscopic properties (UV, IR, NMR, and mass spectra), strongly indicated that its structure was (IV), yet with so far unknown absolute configuration.

Compound B, m.p. 71 – 72°, with the composition $C_7H_{16}N_2OS_2$, was moderately levorotatory and possessed chemical and spectroscopical properties in keeping with structure (V), again with as yet unknown chirality.

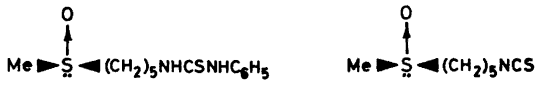
(ii) The remaining part of fraction 2 was treated with triethylamine at room temperature, resulting in slow formation of (IV).

The results strongly indicate that the initial product from the enzymic hydrolysis giving rise to the formation of (IV) and (V) is a chiral 3-hydroxy-

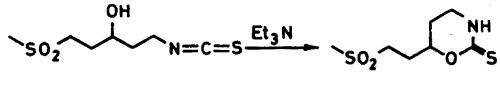


5-methylthiopentyl isothiocyanate (VI). In base-catalyzed reactions (Et_3N), 3-hydroxyalkyl isothiocyanates undergo cyclizations to tetrahydro-1,3-oxazine-2-thiones, as previously established,[4] explaining, in the present case, the formation of (IV) in reaction (ii). In the ammonia reaction (i), two competitive reactions take place, one leading, as expected, to the thiourea (V), and another affording the cyclic derivative (IV).

Fraction 3. The oily residue (155 mg) from fraction 3 possessed properties characteristic for a sulphoxide-isothiocyanate. For further characterization, it was converted into a crystalline phenylthiourea upon reaction with aniline. Analytical and spectroscopical data indicated that the structure of the latter was (VII), confirmed upon critical comparison with an authentic specimen * of (*R*)1-(5-methylsulphinylpentyl)-3-phenylthiourea.[3] Hence, the enzymic hydrolysis product in fraction 3 is (*R*)-5-methylsulphinylpentyl isothiocyanate (VIII), previously encountered as a glucosinolate hydrolysis product in seed extracts of various species of *Alyssum*.[3]

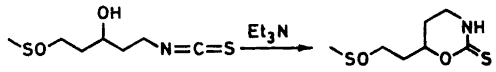


Fraction 4. This fraction (270 mg) contained a crystalline, dextrorotatory isothiocyanate, which according to spectroscopic data, contained a sulphone grouping. On treatment with Et_3N, or heating to 110°, the isothiocyanate cyclized to a chirally still undefined, levorotatory tetrahydro-1,3-oxazine-2-thione possessing the structure (X). Consequently, the isothiocyanate representing the initial enzymic hydrolysis product is 3-hydroxy-5-methylsulphonylpentyl isothiocyanate (IX), the stereochemistry of which has not yet been established.



* The absolute configuration of (VII) has been unequivocally determined by ORD comparison with (*R*)1-(3-methylsulphinylpropyl)-3-phenylthiourea, the structure of which was established by X-ray analysis.[5]

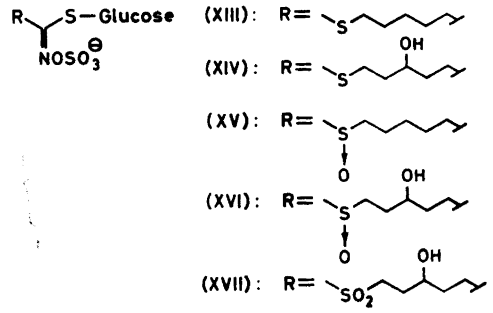
Fraction 5. From this last fraction another levorotatory tetrahydro-1,3-oxazine-2-thione was obtained, subsequent to triethylamine treatment and chromatographic purification. On the basis of composition and spectroscopic properties, it was assigned the structure (XII), undoubtedly resulting from cyclization of an initially formed 3-hydroxy-5-methylsulphinylpentyl isothiocyanate (XI).



(XI) (XII)

DISCUSSION

On the reasonable assumption that the various isothiocyanates and cyclized derivatives discussed above arise by enzymic hydrolysis of glucosinolates in the usual fashion (*cf.* Ref. 6), it is concluded that seed extracts of *E. hieracifolium* L. contain the glucosinolates (XIII) – (XVII), three of which, *viz.* (XIV), (XVI), and (XVII), are new as natural products.



The fact that the glucosinolates (XIII) – (XVII) represent nothing more than oxidation stage variants of the 5-methylthiopentylglucosinolate side-chain (XIII) is noteworthy inasmuch as the methylthiopentyl side-chain is new for the genus *Erysimum.* Seeds of several species previously studied have typically contained glucosinolates with the side-chains $R = CH_3SO_x(CH_2)_y-$, ($x = 0$, 1, 2; $y = 3$ and 4).[1,7,8] Occasionally, other side-chains, such as $MeOOC(CH_2)_3-$,[9,10] (*p*)-$OHC_6H_4CH_2-$,[1] or $CH_2=CHCH_2-$,[1] have been encountered in species of the genus *Erysimum.* The question therefore arises as to the possible significance of chemical characters, such as the glucosinolate composition, for taxonomic purposes within the present group. The genus *Erysimum* constitutes a very complex and polymorphous group of taxa, the classification of which poses great difficulties. This is reflected in an extensive and rather controversial botanical literature on the identity and affinity of the numerous *Erysimum* species, including, *inter alia*, a number of cytotaxonomic studies (*cf.*, *e.g.*, Ref. 11). The observed pattern of glucosinolates in

E. hieracifolium L. is unique within a long series of *Erysimum* species studied so far in this laboratory.[1] The possible taxonomic significance of this observation will be discussed elsewhere in another context.

It would appear likely that the glucosinolates (XIV), (XVI), and (XVII) possessed the same chirality at the carbinol center in the side-chain, resulting, of course, in identical stereochemistry of the derived isothiocyanates and their cyclized counterparts.* Likewise, it would be expected that the sulphoxide-glucosinolate (XVI) possessed the same configuration, *viz.* (*R*), at the chiral sulphoxide center as that prevailing in the co-occurring, non-hydroxylated analogue.[5] Determination of the absolute configurations around the chiral C- and S-centers in the side-chains of the glucosinolates (XIV), (XVI), and (XVII) is presently a matter of study in this laboratory.

EXPERIMENTAL

Melting points are uncorrected and determined in an electrically heated bath. Analytical specimens are dried *in vacuo* over calcium chloride at room temperature before analysis. Paper chromatography is performed on Schleicher & Schüll 2043 b paper. Column chromatography is carried out on Merck 0.05—0.2 mm silicagel. Rotations are measured on a Perkin-Elmer 141 photoelectric polarimeter. UV-spectra are measured on a Perkin-Elmer 402 instrument, IR spectra on a Perkin-Elmer Infracord. NMR-spectra are routinely recorded on a Varian A 60 instrument, and mass spectra on a Perkin-Elmer 270 instrument equipped with a gas chromatograph as well as a solid inlet; ionization potential: 70 eV.

Paperchromatographic analysis. Ground seeds (5 g) of *Erysimum hieracifolium* L. were extracted with hot 70 % MeOH, and the extract was utilized for descending paper chromatography in the solvent systems: (i) butanol:ethanol:water (4:1:4), and (ii) butanol:pyridine:water (6:4:3). The chromatograms were sprayed with silver nitrate, whereby the glucosinolates appeared as grey-brown spots. In (i), major spots were noted with R_B-values (*i.e.* R_F-values relative to that of benzylglucosinolate (I, R=$C_6H_5CH_2$)) of 0.18, 0.46, 0.72, and *ca.* 1.0; in (ii), strong spots were visible with R_B-values of 0.23, 0.45, and *ca.* 1.0. Several of the spots showed signs of being of multicomponent composition.

Enzymic hydrolysis and TLC. An aliquot of the above solution was freed of methanol and subjected to enzymic hydrolysis with a myrosinase preparation. The solution was then extracted with chloroform, and a few µl of the concentrated solution were applied to the corner of a chromatographic silica gel plate. The plate was developed in $CHCl_3$:EtOH (95:5), air-dried, and kept in an NH_3-atmosphere overnight. It was then developed in the same solvent after turning the plate 90°. Spraying with Grote's reagent revealed the presence of at least five thioureas as distinctly blue spots. Three of these were accompanied by more lipophilic products, yielding pale blue colours, quite characteristic for cyclized 2- or 3-hydroxy-substituted alkyl isothiocyanates.

Extraction, enzymic hydrolysis, and separation of products. Seeds of *E. hieracifolium* (300 g) were finely ground and defatted by extraction with two 600 ml-portions of petroleum ether. The seed powder (210 g) was extracted three times with 1.0 l-portions of refluxing 70 % methanol. The extracts were combined, and all methanol was removed by distillation *in vacuo*. The aqueous residue was diluted to 1 l with water, and a 20 % lead acetate solution was added to precipitate impurities. After filtration, Na_2HPO_4-solution was added to remove excess lead ions. The filtrate was brought to pH 6.8, and a myrosinase solution (10 ml), and a trace of ascorbic acid, were added. After 6 h at room temperature, the reaction mixture was extracted five times with 50 ml portions of

* *Added in proof*: CD-measurements have indeed confirmed identical chirality of (IV) and (X).

chloroform. The combined extracts were dried overnight over anhydrous Na_2SO_4 and evaporated to dryness. The residue (2.1 g) was redissolved in a small volume of chloroform and transferred to a column of silicagel (60 g), deactivated with 15 % of water.

The chromatographic development was performed with chloroform (350 ml), followed by 350 ml portions of chloroform containing 1, 2, 4, and 6 % of ethanol. 20 ml fractions were collected.

Fraction 1. This consisted of the first 60 ml of eluate emerging from the column. An aliquot (4 ml) was concentrated on a watchglass. The residue was dissolved in a few drops of ether and injected into a gas-chromatograph combined with a mass spectrometer. One predominant peak appeared, the mass spectrum of which was indistinguishable from that of an authentic specimen of 5-methylthiopentyl isothiocyanate (II).[12]

The remaining solution was treated with methanolic ammonia (25 ml) overnight. The solution was concentrated to dryness, and the residue was recrystallized from ethyl acetate to give a product, which, according to UV-, IR-, and mass-spectrometry, was identical with 1-(5-methylthiopentyl)-thiourea (III).[2]

Fraction. 2. The next 360 ml of the column eluate was evaporated to give about 500 mg of oily material, which was divided into (i) a 300 mg and (ii) a 200 mg portion. These were processed separately as described in the following.

(i) The oily product (300 mg) was dissolved in methanolic ammonia (25 ml) and set aside for 6 h at room temperature. The oily residue was dissolved in a small volume of chloroform and transferred to a silica gel column (50 g). By elution with chloroform, containing an increasing amount of ethanol (from 0 to 8 %), two major fractions were obtained.

The fastest moving component, A, (45 mg) was recrystallized twice from ethyl acetate:petroleum ether to give colourless needles (18 mg), m.p. 58–59°. (Found: C 43.91; H 6.89; N 7.22; S 33.27. Calc. for $C_7H_{13}NOS_2$: C 43.98: H 6.85; N 7.32; S 33.55). $[\alpha]_D^{32}$ −132° (*c* 0.8, abs. EtOH); λ_{max}(EtOH) 253 nm (*ε* 16 000); IR (KBr): strong and characteristic bands at: 3450 (NH), 1560, 1180, and 1160 cm^{-1}. The structure of the product as *(−)-6-(2-methylthioethyl)-tetrahydro-1,3-oxazine-2-thione* (IV) was further supported by the NMR-spectrum (100 MHz), exhibiting signals at δ 8.2 (1H, N*H*), 4.5 (1H, m, −C*H*−O), 3.4 (2H, t, with fine structure, −CH_2−CH_2−NH), 2.7 (2H, t, −S−CH_2−CH_2), 2.18 (3H, s, CH_3−S−), and *ca.* 2.0 (4H, m, −S−CH_2−CH_2−, and −O−CH−CH_2−CH_2−N−). The mass spectrum showed a parent ion at *m/e* 191 and fragments corresponding to loss of 33 (SH) and 47 (CH_3S) mass units; a strong peak at *m/e* 61 was attributable to the stable ion $CH_3SCH_2^+$.

A slower moving fraction, (B), (200 mg) was recrystallized from ethyl acetate: petroleum ether to give nacreous, colourless plates, m.p. 71–72°. (Found: C 40.34; H 7.77; N 13.31; S 30.56. Calc. for $C_7H_{16}N_2OS_2$: C 40.38; H 7.75; N 13.45; S 30.80). $[\alpha]_D^{26}$ −15.7° (*c* 1.6, abs.EtOH); λ_{max}(EtOH) 243 nm (*ε* 11 000); IR (KBr): strong bands at 3300, 1640, 1540, and 1120 cm^{-1}. The structure of the product as *(−)-1-(3-hydroxy-5-methylthiopentyl)-thiourea* (V) was confirmed by the NMR-spectrum (in $(CD_3)_2SO$): δ 7.5 (1H, N*H*), 6.9 (2H, NH_2), 4.5 (1H, d, O*H*), 3.4 (3H, m, −CH_2−N−, and−C*H*OH−), 2.5 (2H, t, −S−CH_2−), 2.0 (3H, s, CH_3S−), and 1.5 (4H, m, −S−CH_2−CH_2−CHOH−, and −CHOH−CH_2−CH_2−N). The mass spectrum contained a molecular ion (*m/e* 208), and fragment ions corresponding to losses of SH (33) and CH_3S (47); again, a strong *m/e* 61 ion ($CH_3SCH_2^+$) was present.

(ii) The 200 mg aliquot of fraction 2, $[\alpha]_D^{25}$ +14° (EtOH), was treated overnight in chloroform solution with a few drops of triethylamine. The solution was evaporated to dryness, redissolved in chloroform, and chromatographed on a silica gel column (10 g) with chloroform:ethanol gradients as the eluting solvent. A crystalline product was obtained and recrystallized from ethyl acetate:petroleum ether, m.p. 58°, slightly lower than but undepressed on admixture with the tetrahydro-oxazinethione (IV) described above. Coinciding IR-spectra further served to confirm the identity.

Fraction 3. The next 120 ml of eluate constituted fraction 3. On evaporation, a colourless oil resulted (155 mg), the mass spectrum of which suggested that it chiefly consisted of 5-methylsulphinylpentyl isothiocyanate (VIII, or the enantiomer).

To the chloroform solution was added aniline (150 mg). Next day, the solution was concentrated, and anhydrous ether was added, causing the separation of colourless

crystals, which were recrystallized twice from ethyl acetate, m.p. 122–123°, $[\alpha]_D^{32}$ −57° (*c* 1.7, EtOH). The identity of the product as *(−)-1-(5-methylsulphinylpentyl)-3-phenylthiourea* (VII) was established upon comparison with an authentic specimen (reported for this:[3] m.p. 126–126.5°, $[\alpha]_D^{25}$ −61.6°±2.5° (*c* 2.14, 96 % EtOH).

Fraction 4. This consisted of the subsequent 180 ml of the eluate. On evaporation, a crystalline residue (270 mg) was obtained. After recrystallization from ethyl acetate: petroleum ether, an analytical specimen (184 mg) was obtained, m.p. 103°, $[\alpha]_D^{27}$ +22° (*c* 1.45, EtOAc). (Found: C 37.62; H 5.81; N 5.84; S 28.65. Calc. for $C_7H_{13}NO_3S_2$: C 37.67; H 5.87; N 6.28; S 28.71). λ_{max}(EtOH) 244 nm (ε 900). The IR-spectrum (KBr) exhibited strong bands at 3500 (OH), 2130–2200 (doublet, NCS), 1300 (SO_2), 1140 (SO_2), and 785 cm^{-1}. The mass spectrum showed a molecular ion at *m/e* 223, and strong peaks at *m/e* 125 (formally corresponding to $CH_2{=}CH{-}CH{=}CH{-}CH_2{-}NCS^+$), and *m/e* 72 ($CH_2NCS^+$) *(vide infra)*. The NMR-spectrum contained signals at δ 4.0 (1H, m, $H_3C{-}CHOH{-}CH_2$), 3.72 (2H, t, $-CH_2{-}CH_2{-}NCS$), 3.24 (2H, t, $CH_2{-}CH_2{-}SO_2-$), 2.99 (3H, s, CH_3SO_2), 2.4 (1H, d, $-CH{-}OH$), and 1.7–2.2 (4H, m, $-CH_2{-}CHOH{-}CH_2-$) ppm. The combined evidence suggests that the compound is *(+)-3-hydroxy-5-methylsulphonylpentyl isothiocyanate* (XI).

In keeping herewith, (XI) underwent cyclization, as followed by UV-spectroscopy, when an alcoholic solution of the isothiocyanate (65 mg) was treated with triethylamine at room temperature overnight. Evaporation to dryness afforded a crystalline residue (47 mg) which was recrystallized twice from ethyl acetate to give an analytical specimen (21 mg), m.p. 143–144°, $[\alpha]_D^{23}$ −92° (*c* 1.1, MeOH). (Found: C 37.80; H 5.95; N 6.15; S 28.26; Calc. for $C_7H_{13}NO_3S_2$: C 37.67; H 5.87; N 6.28; S 28.71). λ_{max}(EtOH) 252 nm (ε 12 300). The IR-spectrum (KBr) was devoid of NCS-absorption, but displayed strong bands at 3 200 (NH), 1560, *ca.* 1300 (SO_2), 1145 (SO_2), 1050, and 795 cm^{-1}. The mass spectrum was virtually identical with that of the corresponding isothiocyanate (XI), suggesting that the latter cyclized thermally in the inlet. The experimental evidence supports a formulation of the compound as *(−)-6-(2-methylsulphonylethyl)-tetrahydro-1,3-oxazine-2-thione* (X).

Attempts to produce a thiourea derivative of (XI) by treatment with methanolic ammonia resulted in isolation of the cyclized product (X). The mother liquors, according to chromatography, seemed to contain the linear thiourea, but no further attempts were made to obtain it in pure form.

Fraction 5. The last fraction, containing 68 mg of solids in 250 ml of eluent, emerged from the column only after prolonged elution with chloroform:ethanol (94:6). Since the composition, according to TLC, proved complex, no attempts were made to isolate the isothiocyanate fraction, but the solution was treated overnight, without further purification, with triethylamine, in order to bring about intramolecular cyclization. The residue (70 mg) was dissolved in a few drops of chloroform and applied to a column, packed with alumina (neutral, 25 g). Elution was performed with 75 ml portions of chloroform, successively containing 1 and 2 % of ethanol; 20 ml fractions were collected. Fractions 5–11 were combined and evaporated to dryness. The residue was recrystallized twice from ethyl acetate in order to obtain an analytical specimen (13 mg), m.p. 136–137° (sintering from 120°), $[\alpha]_D^{28}$ −144° (*c* 1.0, EtOH). (Found: C 38.23; H 6.86; N 6.21. Calc. for $C_7H_{13}NO_3S_2$, 0.5 H_2O: C 38.89; H 6.53; H 6.48). λ_{max}(EtOH) 252 nm (ε 11 300). The infra-red spectrum (KBr) contained strong bands at 3400, 3200, 1640 (H_2O), 1570, 1178, and 1020 (S=O) cm^{-1}, whereas the mass spectrum contained a molecular ion at *m/e* 207, and ionic fragments corresponding to the losses of 17 and 63 (CH_3SO) mass units. The combined evidence indicates that the isolated compound is *(−)-6-(2-methylsulphinylethyl)-tetrahydro-1,3-oxazine-2-thione* (XII). According to the mass spectrum it seems likely that the product is slightly contaminated with the corresponding sulphone (X) described above.

The authors are grateful to Professor R. Gmelin for his help and advice during the present investigation. The invaluable assistance of the Botanic Garden of the University of Copenhagen in the production of large seed quantities of *E. hieracifolium* is greatly appreciated. Thanks are due to Dr. S. Jacobsen for assistance with the mass spectra, and to Dr. C. Pedersen for help with the NMR-spectra.

REFERENCES

1. Gmelin, R., Kjær, A. and Schuster, A. *In preparation.*
2. Kjær, A., Larsen, I. and Gmelin, R. *Acta Chem. Scand.* **9** (1955) 1311.
3. Kjær, A. and Gmelin, R. *Acta Chem. Scand.* **10** (1956) 1100.
4. Kjær, A. and Jensen, R. B. *Acta Chem. Scand.* **12** (1958) 1746.
5. Cheung, K. K., Kjær, A. and Sim, G. A. *Chem. Commun.* **1965** 100.
6. Ettlinger, M. G. and Kjær, A. In Mabry, T. J., Alston, R. E. and Runeckles, V. C. *Recent Advances in Phytochemistry*, Appleton-Century-Crofts, New York 1968, p. 58.
7. Kjær, A. *Fortschr. Chem. Org. Naturstoffe* **18** (1960) 122.
8. Gmelin, R., Kjær, A. and Schuster, A. *Acta Chem. Scand.* **22** (1968) 2875.
9. Kjær, A. and Gmelin, R. *Acta Chem. Scand.* **11** (1957) 577.
10. Gmelin, R. and Kjær, A. *Acta Chem. Scand.* **23** (1969) 2548.
11. Favarger, C. *Bull. Soc. Botan. Suisse* **74** (1964) 5.
12. Kjær, A., Ohashi, M., Wilson, J. M. and Djerassi, C. *Acta Chem. Scand.* **17** (1963) 2143.

Received November 17, 1969.