VOLATILE GLUCOSINOLATE DEGRADATION PRODUCTS OF *BRASSICA NAPUS* AND *SINAPIS ALBA* SEEDS

Suleiman Afsharypour

Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran.

G.Brine Lockwood

Department of Pharmacy, University of Manchester, Manchester, M13, 9PL, England

(Received 15th March 1989)

(Approved 8th January 1990)

ABSTRACT

Brassica napus L. and *Sinapis alba L.* are two cruciferous species for which some medicinal uses are mentioned in the Iranian, Indian and Chinese traditional medicine. Seed oil of *B.napus* has cathartic action, and is either used as such or after partial hydrogenation for cooking, shortening or as margarines. Seeds of *S.alba* have also cathartic action and some other medicinal uses but, because of the recent reports of anticancer or cancer preventitive compounds in the cruciferae family, there is more interest in the nutritional value of crucifers. The anticancer activity of these plants is attributed to the presence of organic sulfur compounds namely the glucosinolates. These glucosides were identified through their volatile degradation products (i.e the aglucones), which are liberated after hydrolysis, using gas-liquid chromatography-mass spectrometry. Other volatile constituents, including some hydrocarbones and fatty acids were identified as well.

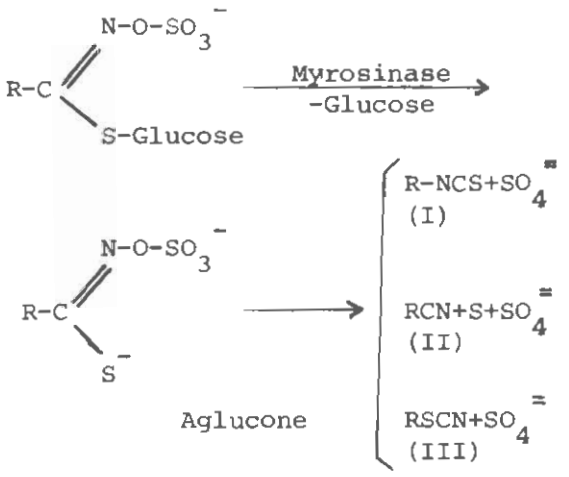
INTRODUCTION

Brassica napus L. (rape seeds) and *Sinapis alba L.* (white mustard) are two members of the Cruciferae family.

These plants are widely cultivated throughout Europe and the Middle East, and have diverse medicinal uses[1]. The anticancer activity of these plants is attributed to the presence of some glucosides called the glucosinolates

[2].Since the aglucones of these glucosides(the mustard oils) are mostly volatile,it was decided to determine the types of glucosinolates present in these plants through determination of their volatile aglucones which are liberated after hydrolysis.

The glucosinolates are thioglucosides found mainly in the Cruciferae family. On hydrolysis they degrade and three main products (i.e: isothiocyanates(I),nitriles(II), and thiocyanates (III) are produced as shown below[3]:



However,within certain crucifers, other degradation products(i.e.:cyanoepithioalkanes, oxazolidinethiones and thionocarbamates)may be produced [4].

The glucosinolates are generally detected through the identification of their degradation products formed on hydrolysis[5].

There are reports indicating that some of the glucosinolates have anti-neoplastic activity through stimulation of mixed function oxidases [2], whilst others have toxic effects on the liver,kidney and thyroid gland [6].Experimentally,it has been found that if glucosionolates,isothiocyanates or nitriles are fed to animals, the uptake of fed radioactive iodine by the thyroid is inhibited[6].

EXPERIMENTAL

Plant material:

Seeds of *B.Napus and S.alba* were obtained from commercial suppliers (Kenneth Wilson Ltd.and Potters Herbal Suppliers Ltd.,Great Britain). Because proper authentication of the seeds was impossible,they were cultivated, and fully developed plants were characterized by the Botany department of Manchester Museum.

Isolation Procedure:

Seeds(30gm of each type) were crushed separately in a coffee mill, then defatted with diethyl ether, dried at room temperature, and weighed. Distilled water(15 times the weight of crushed,defatted and dried material)was then added and the product was covered by a layer of cyclohexane (100 ml) and left for autolysis at 25°C overnight(17 hours). On the next day,each product was shaken for half an hour and the degradation products collected by distillation.

Analysis by Gas Chromatography:

Initially to check the separation

of the components,samples were examined by capillary gas chromatography using a Carlo Erba High Resolution Gas Chromatograph equipped with heated FID*.The Coulumn used was 25m x 0.32 mm id,fused silica bonded OV. 1 (methyl silicone gum). The split ratio was 10:1.The carrier gas was hydrogen with a flow rate of 2ml/minute.Temperature programme was 50 °C initially followed by an increase at 5°C / minute to 280 °C. Injector and detector temperature were 280 °C and 290 °C respectively.Injection volume was 1-2 µl.

Identification of components by Gas Chromatography/Mass Spectroscopy:

A KRATOS Ms 25 instrument was used, equipped with a DS-55 Computer data output.The same coulumn and gas chromatography conditions as used in the case of capillary gas chromatography, but with helium as carrier gas . The all glass jet separator interface operated at 250°C.

Mass spectrometer conditions were:ionization potential,70 ev;ionization current,300 A;source temperature,250°C ;resolution,600;scan speed, 1 second/decade.

RESULTS AND DISCUSSION

The M^+ of the eight main fragment ions and their relative intensities as well as the elucidated identity of the volatile aglucones of glucosinolates and other volatile substances of crushed autolized seeds of *B.napus* and *S.alba*, are shown in tables 1 and 2 respectively. It should be mentioned that the volatile components are listed in their order of separation. The presence of the hydrocarbons and the fatty acids in the distillates of both species has been further identified by co-chromatography with authentic standards using gas chromatography.

The detected volatile aglucones in *B.napus* are produced from the hydrolysis(autolysis)of 3- butenyl, isohexyl,4-methylthiobutyl,2-phenylethyl,isoheptyl and 5-methylthiohexyl glucosinolates. While in *S.alba*, the detected isothiocyanate,namely benzylisothiocyanate is the hydrolysis product of benzyl glucosinolate. Table 3 shows the structural formulae of the detected glucosinolate degradation products and the related glucosinolates in *B.napus* and *S.alba* seeds.

REFERENCES

1 .Duke,J.A.and Dyensu,E.S.," Medicinal Plants of China",Vol.1,p.206 , Reference Publication Inc.,Michigan(1980).

2 .Wattenberg,L.W.,*J.Nat.Cancer Inst.*, 60,11(1978).

3 .Macleod,A.J.,Panesar,S.S.and Gill, V.,*Phytochemistry* 20,977(1981).

4 .Vaughan,J.G.,Macleod,A.J.and Jones , B.M.G.,"The Biology and Chemistry of the Cruciferae",p.45,Academic Press,London(1976).

*Flame Ionization Detector

5 .Harborne,J.B.,Phytochemical Methods, p.172, Chapman and Hall , London (1984).

6 .Van Etten,C.H.and Tookey ,H.L., Herbivores:Their Interaction With Secondary Plant Metabolites,Rosenthal,G.A.,*et al* eds.,p.471,Academic Press,New York(1979).

Table 1 -Identified distillate products obtained after autolysis of *B.napus* seeds

No	M^+(%)	Eight main fragment ions (%)	Identity
1	113(82)	27(100),113(82),55(69), 39(64),41(48),27(48), 56(46),85(37).	3-Butenyl isothio - cyantate.
2	143(27)	43(100),41(67),56(40), 27(40),128(37),143(27), 69(27),110(20).	Isohexyl Isothio - cyanate
3	136(100)	136(100),135(98),77(29), 90(26),79(22),61(16), 107(16),89(14).	2-Hydroxy- 3-Methyl benzaldehyde
4	129(33)	61(100),129(33),82(25), 55(20),47(14),48(26), 45(13),54(10).	5-Methyl - thiopenta nitrile
5	131(17)	91(100),55(70),41(46), 39(28),131(17),65(12), 51(10),27(10).	3-Phenylpro - prio nitrile
6	157(6)	43(100),124(96),41(95), 55(86),115(57),72(50), 57(37),56(35).	Isoheptyl isothio - cyanate

Continued table 1

No	M^+ (%)	Eight main fragment ions (%)	Identity
7	143(26)	61(100),55(29),143(26), 96(22),41(21),69(15), 47(11),97(10).	6-Methylthio-hexanitrile
8	184(2)	57(100),43(84),71(52), 41(39),85(30),55(18), 56(15),29(14).	n-Tridecane
9	163(38)	91(100),163(38),65(21), 105(20),77(16),92(15), 51(14),39(10).	2-Phenylethyl isothiocyanate
10	212(0)	57(100),43(90),71(68), 85(49),61(47),41(46), 56(37),55(25).	n-Pentadecane*
11	256(26)	73(100),43(88),60(80), 57(79),55(77),41(67), 69(50),98(50).	Palmitic acid
12	282(2)	55(100),69(71),41(67), 83(65),97(49),43(48), 67(44),81(40).	Oleic acid

*This n-alkane was further identified by co-chromatoraphy with an authentic standard .

Table 2 -Identified distillate products obtained after autolysis of *S.alba* seeds

No	M^+(%)	Eight main fragment ions (%)	Identity
1	-	57(100),43(98),91(52), 71(37),41(37),119(35), 92(25),133(23).	A hydrocarbon
2	148(100)	148(100),147(51),77(25), 133(26),105(24),117(23), 115(19),121(18).	Anethole
3	184(2)	57(100),43(87),71(51), 41(34),85(32),55(16), 56(15),42(10).	n-Tridecane
4	149(14)	91(100),65(18),149(14), 92(11),39(8),63(7), 51(6),89(6).	Benzyl isothio-cyanate
5	204(3)	41(100),69(97),93(88), 79(58),55(57),91(52), 133(48),81(46).	β-Caryophyllene
6	198(0)	57(100),43(90),71(56), 41(38),85(26),55(18), 73(16),29(16).	n-Tetradecane*
7	220(28)	205(100),220(28),57(16), 206(14),145(12),105(7), 41(6),81(6).	4-Methyl-2,6-ditertbutyl phenol
8	212(0.5)	57(100),43(81),71(63), 85(38),41(34),69(22), 55(19),56(14).	n-Pentadecane*
9	200(5)	73(100),60(89),57(84), 43(82),41(61),55(54), 71(51),85(38).	Lauric acid

Continued table 2

No	M^+ (%)	Eight main fragment ions (%)	Identity
10	228(9)	73(100),60(81),43(65), 55(52),41(40),57(39), 129(27),69(27).	Myristic acid
11	256(14)	73(100),60(78),43(77), 57(63),55(52),41(47), 71(32),68(29).	Palmitic acid
12	282(2)	55(100),41(76),69(65), 43(50),83(45),67(41), 81(33),97(31).	Oleic acid

*These n-alkanes were further identified by co-chromatography with authentic standards

Table 3 -Names,Structural formulae of the identified glucosinolate degradation products,and the related glucosinolates in *B. napus* and *S.alba* seeds.

Identitied glucosinolate degradation products	Structural formulae	Related glucosinolates
3-Butenyl isothiocyanate	$CH_2=CH(CH_2)_2NCS$	3-Butenyl
Isohexyl isothiocyanate	$(CH_3)_2-CH(CH_2)NCS$	Isohexyl
5-Methylthiopentanitrile	$CH_3-S(CH_2)_4-CN$	4-methylthio-butyl
3-Phenylpropionitrile	$C_6H_5-(CH_2)-CN$	2-Phenylethyl
2-Phenylethyl isothio - cyanate	$C_6H_5-(CH_2)-NCS$	
Isoheptyl isothiocyanate	$(CH_3)_2-CH(CH_2)_4NCS$	Isoheptyl -
6-Methylthiohexanitrile	$CH_3S(CH_2)_5CN$	5-Methylthio-pentyl
Benzyl isothiocyanate	$C_6H_5-CH_2NCS$	Benzyl