

Glucosinolates and Derived Products in Cruciferous Vegetables. Analysis of the Edible Part from Twenty-Two Varieties of Cabbage

Cecil H. VanEtten,* Melvin E. Daxenbichler, Paul H. Williams, and William F. Kwolek

Knowledge of glucosinolate (GS) content in cabbage (*Brassica oleracea*) is needed to establish levels at which these compounds or their enzymically released products are consumed. New methods of analysis for individual and total GS were applied to 12 open-pollinated and 10 hybrid varieties. Total glucosinolates in cabbage as harvested ranged from 299 to 1288 ppm. Of the 12 GS's determined, those found in the greatest amount were: two 3-indolylmethyl GS's (18 to 63 ppm as thiocyanate ion); allyl GS [4 to 146 ppm as allyl isothiocyanate (ITC)]; 3-methylsulfinylpropyl GS (30 to 164 ppm as ITC); 4-methylsulfinylbutyl GS (0.2 to 119 ppm as ITC). The last two have not been quantitated previously in edible cabbage. Goitrin content ranged from 1.2 to 26 ppm. Significant differences among varieties were found and are attributed to genetic variability. Variation in GS content from head to head within a variety was greater in the open-pollinated than in the hybrid varieties.

There is concern that new plant varieties may conceivably contain larger amounts of natural toxicants than their progenitors (Hanson, 1974). In the Cruciferae family of which cabbage is a member, glucosinolates are present which are the source of undesirable and even harmful substances if ingested in large amounts (VanEtten and Wolff, 1973). To evaluate new varieties for glucosinolate content, more detailed information is needed as to kinds and amounts of glucosinolates in current domestic cabbages. As determined by new analytical methods, reported here are quantitative analyses for total and specific glucosinolates in selected varieties. The results confirm and extend previous knowledge of these substances and their hydrolysis products.

EXPERIMENTAL SECTION

Plan. To provide a base for statistical treatment, 132 analyses were made on 110 heads. These heads were randomly selected from each of 22 varieties and grouped into 5 sets—1 medium, 2 large, and 2 small heads from each variety. The large and small heads were each analyzed once, but each medium-sized head was analyzed in duplicate.

Source and Preparation of Samples. The varieties (12 open-pollinated and 10 hybrids) were grown on adjacent plots at the University of Wisconsin, Madison. The heads were harvested in mid-October 1974 and refrigerated until preparation of the analytical sample.

Within a month, extracts were made as follows from each head and were immediately frozen. A 100-g sample was cut as a wedge with the edge parallel to the core. The sample was cut into 1 to 3 cm cubes and dropped into boiling methanol (300 ml analytical grade) on a steam bath in a 1-l. wide-mouthed Erlenmeyer flask covered with a watch glass. Methanol was replaced as needed while the mixture was boiled for 15 min. After cooling, the material was finely dispersed with a blender and vacuum filtered. The residue was washed once with methanol, then resuspended in 70:30 methanol/water, heated, cooled, and

filtered a second time followed by a final wash with 70:30 methanol/water. The combined filtrates were reduced to ca. 25 ml under vacuum at <50 °C. The concentrate was centrifuged, and the supernate was diluted to 100 ml and frozen.

Methods of Analysis. Within 4 months after the extracts were frozen, they were analyzed for total and individual glucosinolate content by unpublished procedures developed at this laboratory (VanEtten and Daxenbichler, 1975; Daxenbichler and VanEtten, 1975). Briefly the methods are as follows. The glucosinolates in an aliquot of the cabbage extract are adsorbed on an anion exchange resin (Dowex 1-X2). The resin/glucosinolate complex is then shaken overnight with an aqueous myrosinase preparation (pH 7) and methylene chloride. Total glucosinolate in the extract is estimated from the glucose that is released into the water phase of the hydrolyzing mixture. Individual glucosinolates are estimated by gas-liquid chromatography (GLC) analysis of organic isothiocyanates and goitrin that are extracted into the dichloromethane phase of the hydrolyzed mixture. Identification and quantitation are based on dual glass columns, temperature programmed, GLC analyses. The stationary phases used, 1% EGSS-X and 3% Apiezon L on Gas-Chrom Q, are sufficiently different in polarity to provide adequate differences in elution times as a means for component identification when compared with standard mixtures. The two glucobrassicins were measured together from the thiocyanate ion formed by myrosinase hydrolysis on a separate aliquot of the cabbage extract by a procedure similar to that reported by Josefsson (1968) for the analysis of sinalbin in *Sinapis alba*.

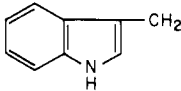
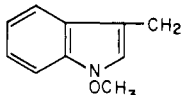
RESULTS AND DISCUSSION

Identification of Glucosinolates. Glucosinolates and their associated hydrolysis products determined in this study are given in Table I. Of the 12 glucosinolates, 10 were previously found in cabbage by Josefsson (1970). Bailey et al. (1961) have tentatively identified benzyl glucosinolate in fresh material. The twelfth glucosinolate tentatively identified here as 4-methylsulfonylbutyl glucosinolate (glucoerysolin) has not been reported previously.

Amounts of Glucosinolates. Data in Table II are grouped to enable comparison of results for open-pollinated and hybrid varieties, and for small and large head size as well as to illustrate the precision of the method as tested with duplicate analyses on each medium-sized head. Glucosinolate content is not significantly different for the open-pollinated varieties vs. the hybrid varieties.

Northern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604 (C.H.V., M.E.D.), Department of Plant Pathology, College of Agricultural and Life Sciences and Agricultural Experimental Station of University of Wisconsin, Madison, Wisconsin 53706 (P.H.W.), and Biometrician, North Central Region, Agricultural Research Service, U.S. Department of Agriculture, stationed at the Northern Laboratory (W.F.K.).

Table I. Types of Glucosinolates in Edible Cabbage

$\begin{array}{c} \text{Glucosinolate} \\ \text{SC}_6\text{H}_{11}\text{O}_5 \\ \text{R}-\text{C} \\ \text{NOSO}_2\text{O}^-\text{K}^+ \end{array}$		R group	Nonchemical name	Part measured ^d
Allyl	$\text{CH}_2=\text{CHCH}_2$	Sinigrin	Allyl NCS; volatile mustard oil	
3-Methylthiopropyl	$\text{CH}_3\text{S}(\text{CH}_2)_3$	Glucobervirin	3-Methylthiopropyl NCS; volatile mustard oil	
3-Methylsulfinylpropyl	$\text{CH}_3\text{SO}(\text{CH}_2)_3$	Gluciberin	3-Methylsulfinylpropyl NCS; nonvolatile mustard oil	
Butenyl	$\text{CH}_2=\text{CH}(\text{CH}_2)_2$	Gluconapin	3-Butenyl NCS; volatile mustard oil	
(<i>R</i>)-2-Hydroxy-3-butenyl	$\text{CH}_2=\text{CHCHOHCH}_2$	Progoitrin	(<i>S</i>)-5-Vinylloxazolidinethione; goitrin	
4-Methylthiobutyl	$\text{CH}_3\text{S}(\text{CH}_2)_4$	Glucorucin	4-Methylthiobutyl NCS; volatile mustard oil	
4-Methylsulfinylbutyl	$\text{CH}_3\text{SO}(\text{CH}_2)_4$	Glucoraphanin	4-Methylsulfinylbutyl NCS; nonvolatile mustard oil	
4-Methylsulfonylbutyl	$\text{CH}_3\text{SO}_2(\text{CH}_2)_4$	Glucoerysolin	4-Methylsulfonylbutyl NCS; nonvolatile mustard oil	
Benzyl	$\text{C}_6\text{H}_5\text{CH}_2$	Glucotropaeolin	Benzyl NCS; volatile mustard oil	
Phenylethyl	$\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2$	Gluconasturtiin	Phenylethyl NCS; volatile mustard oil	
3-Indolylmethyl		Glucobrassicin	Thiocyanate ion; SCN ⁻	
<i>N</i> -Methoxy-3-indolylmethyl		Neoglucobrassicin	Thiocyanate ion; SCN ⁻	

^a Organic isothiocyanates (NCS) may be called mustard oils; volatile mustard oils can be distilled with steam; the non-volatile ones cannot. Estimations of the amounts of each present are made by GLC. The two indolylmethyl glucosinolates are estimated together from the amount of thiocyanate ion formed by hydrolysis. The compounds are grouped as three aglucons that are propyl derivatives, five that are butyl, two that are benzyl, and two (estimated together as thiocyanate ion) that are 3-indolylmethyl derivatives.

Table II. Glucosinolate Content of 12 Open-Pollinated and 10 Hybrid Varieties of Cabbage^a

Product determined	Open pollinated, mean \pm SD, ppm			Hybrid, mean \pm SD, ppm		
	Small ^b	Large ^b	Medium ^c	Small ^b	Large ^b	Medium ^c
Allyl isothiocyanate (NCS) ^d	35.6 \pm 27.8	24.5 \pm 14.1	23.3 \pm 4.3	48.5 \pm 12.7	33.4 \pm 10.9	40.9 \pm 8.2
3-Methylthiopropyl NCS ^d	0.4 \pm 0.6	0.8 \pm 0.9	0.4 \pm 0.2	0.6 \pm 0.7	0.9 \pm 0.8	0.8 \pm 0.4
3-Methylsulfinylpropyl NCS	61.3 \pm 23.7	56.4 \pm 46.7	74.9 \pm 3.8	55.9 \pm 15.6	45.2 \pm 12.1	55.3 \pm 4.0
Butenyl (NCS) ^d	6.1 \pm 8.5	4.9 \pm 7.3	3.1 \pm 1.0	3.6 \pm 3.2	3.4 \pm 3.3	3.5 \pm 0.8
5-Vinylloxazolidinethione (Goitrin)	7.5 \pm 5.6	5.6 \pm 5.5	5.4 \pm 2.0	8.5 \pm 3.2	6.3 \pm 6.9	6.7 \pm 0.8
4-Methylthiobutyl NCS ^d	0.2 \pm 1.1	0.6 \pm 0.8	0.1 \pm 0.3	0.2 \pm 0.1	0.7 \pm 1.1	0.2 \pm 0.1
4-Methylsulfinylbutyl NCS	21.6 \pm 35.4	30.0 \pm 20.8	23.4 \pm 2.7	16.6 \pm 4.9	14.3 \pm 11.7	12.2 \pm 1.0
4-Methylsulfonylbutyl NCS	5.7 \pm 2.7	5.9 \pm 3.8	6.6 \pm 2.8	5.2 \pm 2.6	4.4 \pm 3.0	4.4 \pm 1.0
Benzyl NCS ^d	0.5 \pm 0.5	0.4 \pm 0.5	0.5 \pm 0.1	1.0 \pm 0.5	0.4 \pm 0.4	0.8 \pm 0.1
Phenylethyl NCS ^d	2.3 \pm 1.6	2.4 \pm 2.1	2.4 \pm 0.3	2.0 \pm 0.7	2.0 \pm 0.6	2.0 \pm 0.3
Thiocyanate ion SCN ⁻	38.5 \pm 20.7	26.6 \pm 10.1	33.3 \pm 3.9	30.3 \pm 9.2	23.9 \pm 6.1	29.0 \pm 3.5
Total glucosinolate ^e	850.0 \pm 302.0	658.0 \pm 355.0	730.0 \pm 62.0	735.0 \pm 150.0	587.0 \pm 118.0	663.0 \pm 31.0
Glucosinolates as aglucons, %	93.0 \pm 11.8	96.0 \pm 8.2	97.0 \pm 10.4	96.0 \pm 11.3	98.0 \pm 8.0	100.0 \pm 8.6

^a Expressed as parts per million as harvested (when compared with other methods, 50 ppm = 0.005% = 5 mg per 100 g); SD = standard deviation. Aglucons calculated as isothiocyanates from mustard oil forming glucosinolates, goitrin from progoitrin, and thiocyanate ion from glucobrassicins. Ratios of SD exceeding 1.85 (open pollinated) or 1.93 (hybrid) are significant. ^b Data calculated from single analyses on two small and two large heads. ^c Data calculated from two analyses that included two samples from one medium-sized head, which measures the precision of the method. ^d Volatile mustard oils. ^e Total glucosinolate calculated from glucose released by myrosinase using the average mol wt 457 of the potassium salts of allyl, 3-methylsulfinylpropyl, 4-methylsulfinylbutyl, and 3-indolylmethyl (glucobrassicin), which were the major glucosinolates found.

Head to head variation, for both large and small heads, is greater for the open-pollinated varieties, as noted by the standard deviation (SD). Total glucosinolate content within each variety tends to be inversely proportional with head size.

The SD value on the medium-sized head is computed from the duplicate analyses. Expressed as relative

standard deviation, the precision of the method is $\pm 6.6\%$ for total glucosinolates. For the major isothiocyanates the relative standard deviation is: allyl, $\pm 19.2\%$; for 3-methylsulfinylpropyl, $\pm 6.0\%$; and for 4-methylsulfinylbutyl, $\pm 9.9\%$. For thiocyanate ion from the 3-indolylmethyl glucosinolates the relative standard deviation is $\pm 11.8\%$. The poorer precision for allyl isothiocyanate is

Table III. Amount of Glucosinolates in Edible Part of 22 Varieties of Cabbage^a

Variety	Av ^b head wt, kg	3-Carbon aglucons				4-Carbon aglucons				Benzyl aglucons		Indolyl aglucons, SCN ion, ppm	Total, gluco- sinolates, ppm	Total gluco- sinolates calcd from gluco- sinolates, % %		
		Allyl- NCS, ppm	Methyl- thio- propyl- NCS, ppm	Methyl- sulfinyl- propyl- NCS, ppm	Butenyl- NCS, ppm	Methyl- thio- butyl- NCS, ppm	Methyl- sulfinyl- butyl- NCS, ppm	4- Methyl- sulfonyl- butyl- NCS, ppm	Benzyl- NCS, ppm	Phenyl- ethyl- NCS, ppm						
											Goitrin, ppm				Methyl- butyl- NCS, ppm	Benzyl- NCS, ppm
From 12 Open-Pollinated Varieties																
Badger Market	0.7	40.0	1.4	61.3	2.8	7.7	0.1	7.7	0.1	5.2	3.7	1.9	2.6	18.2	595.0	98.0
Copenhagen Market	1.0	34.0	0.6	61.2	1.6	3.0	0.1	3.0	0.1	5.5	3.2	0.4	2.5	20.0	565.0	93.0
Early Round Dutch	1.0	26.9	0.2	46.0	1.5	4.3	0.0	4.3	0.0	2.9	8.2	0.7	1.3	26.8	525.0	99.0
Globe	1.3	58.4	0.6	81.4	4.1	6.8	0.0	6.8	0.0	11.7	4.0	0.1	2.2	20.5	783.0	97.0
Golden Acre	0.7	18.0	0.6	31.6	0.2	1.4	0.0	1.4	0.0	1.4	1.2	0.0	0.7	15.8	299.0	105.0
Jersey Queen	0.7	4.2	0.2	43.4	0.2	1.7	0.1	1.7	0.1	13.2	4.9	0.0	1.7	24.2	418.0	95.0
Penn State Ball Head	0.6	30.7	0.6	82.7	1.8	3.5	0.0	3.5	0.0	15.1	7.7	1.7	3.3	39.8	866.0	92.0
Red Acre	0.5	13.9	0.0	20.1	18.4	18.3	1.2	18.3	1.2	82.7	4.8	0.2	1.1	24.0 ^c	701.0	94.0
Red Hollander	0.4	11.0	0.1	32.0	15.6	12.9	1.3	15.6	1.3	118.6	9.2	0.0	1.4	58.0 ^c	1203.0	87.0
Round Dutch	1.2	30.5	0.6	60.7	8.0	9.9	0.5	9.9	0.5	24.9	2.6	0.8	1.6	24.0	675.0	95.0
Savoy Perfected	1.4	35.5	0.8	163.8	1.0	2.0	0.2	2.0	0.2	12.2	10.2	0.0	5.5	62.8	1288.0	95.0
Drumhead																
Wisconsin Hollander	0.7	30.5	0.2	86.0	1.4	2.3	0.0	2.3	0.0	6.9	12.7	0.1	6.1	60.0	1014.0	92.0
Approximate LSD ^d		21.9	0.8	36.5	7.8	5.6	1.0	5.6	1.0	28.6	3.8	0.5	1.8	16.2	131.0	12.0
From 10 Hybrid Varieties																
Badger Hybrid 15	1.0	29.5	1.7	51.9	3.4	9.2	0.6	9.2	0.6	15.2	3.2	0.7	1.2	21.7	539.0	104.0
Excel	1.2	19.3	0.7	54.5	1.1	1.7	0.1	1.7	0.1	5.4	9.6	0.0	2.7	41.3	590.0	105.0
Green Boy	0.7	29.7	0.4	73.2	1.4	4.6	0.0	4.6	0.0	8.4	4.0	1.4	2.4	31.7	687.0	97.0
Harvest Queen	0.6	14.3	0.1	31.0	12.0	26.4	0.6	26.4	0.6	51.2	6.2	0.5	2.6	24.3	670.0	103.0
Head Start	0.9	42.8	0.3	55.9	1.8	3.2	0.0	3.2	0.0	4.2	1.8	0.1	2.2	20.2	550.0	94.0
Hercules	1.4	28.7	1.0	42.7	0.4	1.2	0.4	1.2	0.4	1.9	4.0	0.1	1.0	30.8	528.0	97.0
Jet Pak	0.9	31.4	1.5	52.6	2.5	3.0	0.2	3.0	0.2	10.1	2.4	0.3	1.1	22.7	530.0	96.0
Market Prize	1.1	5.7	0.3	22.6	4.6	12.0	1.4	12.0	1.4	34.8	6.2	0.3	0.9	29.2	477.0	105.0
Sanibel	0.8	62.0	0.8	115.6	4.2	5.6	0.0	5.6	0.0	12.4	4.8	2.8	2.5	24.8	990.0	90.0
Stonehead	0.7	145.8	1.0	21.2	3.8	4.6	0.2	4.6	0.2	0.2	4.2	1.2	3.4	30.8	1065.0	90.0
Approximate LSD ^d		13.0	0.8	14.0	3.2	5.3	0.8	5.3	0.8	8.9	2.9	0.5	0.7	8.0	53.2	11.0

^a Mean results in parts per million as harvested from single analyses on two large and two small heads and duplicate analysis on one medium-sized head. All are market varieties; Harvest Queen, Sanibel, and Wisconsin Hollander are suitable also for kraut; Wisconsin Hollander and Excel store well. ^b Mean weight of heads sampled. ^c Estimated by difference between total glucosinolates from glucose and glucosinolates from remaining aglucons. Pigments in the extract interfered with colorimetric measurement of thiocyanate ion. ^d LSD = least significant difference between varieties at the 95% level based on variation between heads.

Table IV. Correlations of Glucosinolate Types with Cabbage Varieties

Group	Open pollinated	Hybrids
A	Badger Market, Copenhagen Market, Early Round Dutch, Globe, Golden Acre, Penn State Ball Head, Round Dutch	Badger Hybrid 15, Green Boy, Head Start, Hercules, Jet Pak, Sanibel
B	Jersey Queen, Savoy Perfected Drum, Head, Wisconsin Hollander	Excel
C		Stonehead
D	Red Acre, Red Hollander	Market Prize, Harvest Queen

probably caused by variable losses due to the compound's volatility.

Total glucosinolate by glucose estimation agrees well with the total calculated by summing estimates of organic isothiocyanates, goitrin, and the thiocyanate ion as glucosinolates (Table II). This indicates that no major glucosinolates of the 12 measured are greatly in error. However, significant errors in the quantities of the minor glucosinolates would have little effect on the total glucosinolate level.

Prior quantitative data on glucosinolates in edible cabbage are limited to indirect estimations as thiocyanate ion, goitrin, and volatile mustard oils as a group. The data given here are in good agreement with values reported by Langer (1964) on 24 samples for these three groups and by Sedlak (1965) on 84 samples for volatile mustard oils. Data reported by Josefsson (1967) for the three groups also agree except that larger amounts of thiocyanate ion were found in four of the eight varieties he analyzed.

Amount and Kind of Glucosinolates as Related to Varieties. There are marked differences among many of the open-pollinated varieties and the hybrids (Table III) both as to kind of glucosinolate and total amount. The correlations between all possible pairs of varieties were computed using amounts of the 11 glucosinolates. Varieties were then grouped based on the magnitudes of these correlations. Varieties from the same group tended to have higher correlations among themselves than with any other varieties. The four groups established by this procedure are shown in Table IV. Groups A, B, and C contain more total glucosinolates from 3-carbon (propyl) than from 4-carbon (butyl) aglucons. In group D the 4-carbon aglucons predominate. Group C (Stonehead) differs in that it is very high in the 3-carbon allyl glucosinolate but low in both 3- and 4-carbon methylsulfinyl glucosinolates. Groups A and B have similar patterns. In group A the correlations are slightly greater than within group B. Group D varieties, which release the largest amount of goitrin, are highly correlated between themselves but are generally not correlated with all other varieties. Stonehead, the only variety in group C, has low nonsignificant correlation with all others. Such correlations may be important, because they may be directly related to genetic stock and could serve as a guide to future plant breeding.

Little information was found on total glucosinolate content of fresh cabbage. Tables II and III report large

amounts of nonvolatile mustard oil which previously has not been reported quantitatively in the literature. In view of the amounts of these compounds found in *B. oleracea* seed by Ettliger and Thompson (1962), it is not surprising to find them in the fresh vegetable.

Literature on the biological effect of products from the glucosinolates in Cruciferous vegetables has been confined mainly to volatile mustard oils, goitrin, and thiocyanate ion (VanEtten and Wolff, 1973). In view of the large amounts of 3-methylsulfinylpropyl and 4-methylsulfinylbutyl isothiocyanates found in cabbage, these substances should also be studied. Also of concern is that organic nitriles, which have a different biological effect, are often formed instead of mustard oils and goitrin when leafy material or seeds are autolyzed (Daxenbichler et al., 1975; Kirk and MacDonald, 1974; VanEtten and Daxenbichler, 1971; VanEtten et al., 1969).

Variation in glucosinolate composition of different varieties grown under the same conditions indicates genetic variability. Thus, it appears new varieties may be developed that contain no progoitrin. However, substitution of one or more glucosinolates for others that are sources of compounds of unknown biological effect may be less satisfactory than the replaced glucosinolate. The relationship of cabbage flavors to glucosinolate content and the breeding for improved flavor should be investigated.

ACKNOWLEDGMENT

We thank William P. Schroeder for technical assistance.

LITERATURE CITED

- Bailey, S. D., Bazinet, M. L., Driscoll, J. L., McCarthy, A. I., *J. Food Sci.* **26**, 163 (1961).
- Daxenbichler, M. E., Spencer, G. F., VanEtten, C. H., Northern Regional Research Laboratory, Peoria, Ill., unpublished data, 1975.
- Daxenbichler, M. E., VanEtten, C. H., Northern Regional Research Laboratory, Peoria, Ill., unpublished data, 1975.
- Ettliger, M. G., Thompson, C. P., Department of Chemistry, Rice Institute, Houston, Tex., Final Report on Contract DA-19-129-QM-1689 to Quartermaster Research and Engineering Command, Natick, Mass., 1962.
- Hanson, C. H., Ed., "The Effect of FDA Regulations (GRAS) on Plant Breeding and Processing", Special Publication No. 5, Crop Science Society of America, Madison, Wis., 1974.
- Josefsson, E., *J. Sci. Food Agric.* **19**, 192 (1968).
- Josefsson, E., *Phytochemistry*, **6**, 1617 (1967).
- Josefsson, E., "Pattern, Content, and Biosynthesis of Glucosinolates in Some Cultivated Cruciferae", Division of Chemistry, Swedish Seed Association, Svalof, Sweden, 1970.
- Kirk, J. T. D., MacDonald, C. G., *Phytochemistry* **13**, 2611 (1974).
- Langer, P., *Physiol. Bohemoslov.* **13**, 542 (1964).
- Sedlak, J., *Biologia (Bratislava)* **20**, 100 (1965).
- VanEtten, C. H., Daxenbichler, M. E., *J. Agric. Food Chem.* **19**, 194 (1971).
- VanEtten, C. H., Daxenbichler, M. E., Northern Regional Research Laboratory, Peoria, Ill., unpublished data, 1975.
- VanEtten, C. H., Gagne, W. E., Robbins, D. J., Daxenbichler, M. E., Wolff, I. A., *Cereal Chem.* **46**, 145 (1969).
- VanEtten, C. H., Wolff, I. A., "Toxicants Occurring Naturally in Foods", 2nd ed, Committee on Food Protection, National Academy of Sciences, Washington, D.C., 1973, p 210.

Received for review November 24, 1975. Accepted February 13, 1976.