Pergamon

Tetrahedron 54 (1998) 3549–3566

TETRAHEDRON

New Syntheses of Indole Phytoalexins and Related Compounds

Peter Kutschy[a*], Milan Dzurilla[a], Mitsuo Takasugi[b], Marcel Török[a], Ingrid Achbergerová[a], Renáta Homzová[a] and Mária Rácová[a]

[a]Department of Organic Chemistry, Faculty of Natural Sciences, P. J. Šafárik University, Moyzesova 11, SK-041 67 Košice, Slovak Republic

[b]Division of Material Science, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060, Japan

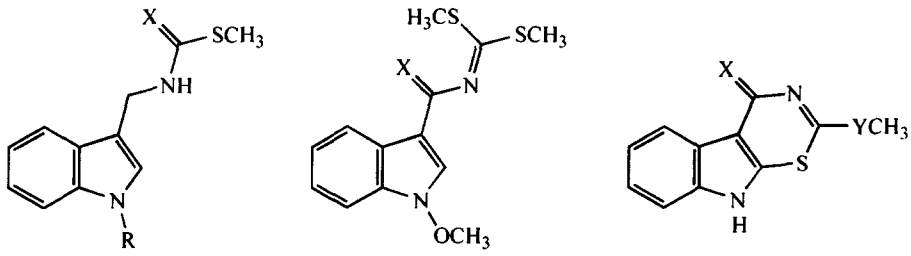
Received 11 November 1997; revised 26 January 1998; accepted 29 January 1998

Abstract: Synthesis of indole phytoalexins brassinin, brassitin, cyclobrassinin and related compounds *via* 3-aminomethylindole and its 1-substituted derivatives obtained by nickel boride catalyzed reduction of corresponding aldoximes with sodium borohydride and *via* [1-(*t*-butoxycarbonyl)-indol-3-yl]methyl isothiocyanate, the first stable derivative of indol-3-ylmethyl isothiocyanate is described. Antifungal activity of the prepared compounds was examined by using the fungus *Bipolaris leersiae* by t.l.c. bioassay and quantitative screening was carried out with the selected compounds.

INTRODUCTION

Phytoalexins are antimicrobial low molecular weight secondary metabolites, produced by plants after their exposure to biological, chemical, or physical stress.[1] These compounds play an important role in the investigation of defence mechanisms of plants against microbial pathogens.[2-6] During the last decade, more than twenty phytoalexins have been isolated from the plant family Cruciferae, including many economically important vegetables.[7-12] Common structural feature of cruciferous phytoalexins is the presence of indole nucleus and a side chain or another heterocycle, containing an atom of nitrogen and one or two sulfur atoms. Typical representatives of these compounds (Figure 1) were isolated from Chinese cabbage {[(brassinin (**1**), methoxybrassinin (**2**), cyclobrassinin (**7**)][11,12] and methoxybrassitin (**4**)[13]}, Japanese radish [brassitin (**3**)[10] and spirobrassinin (**9**)[14]], cabbage [methoxybrassenin A (**5**) and methoxybrassenin B (**6**)],[15] kohlrabi [cyclobrassinon (**8**) and methoxyspirobrassinin (**10**)],[9] false flax [camalexin (**11**)][16] and Indian mustard [brassilexin (**12**)].[17] Biosynthetic studies on brassinin, cyclobrassinin and spirobrassinin revealed that their biosynthesis proceeds from L-tryptophan, which is *via* indole glucosinolate glucobrassicin and/or directly . e.g. *via* thiohydroxamic acid, transformed to indol-3-ylmethyl isothiocyanate as the key biosynthetic intermediate, undergoing further transformation to indole phytoalexins.[18-20] Indol-3-ylmethyl isothiocyanate was detected by trapping experiment with sodium methane thiolate, resulting in the isolation of brassinin.[19]

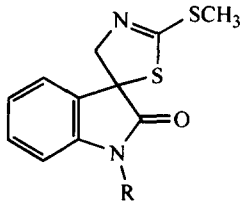
0040-4020/98/$19.00
PII: S0040-4020(98)00088-X



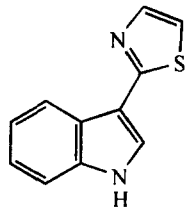
1, R = H, X = S
2, R = OCH_3, X =S
3, R = H, X = O
4, R = OCH_3, X = O

5, X = H, H
6, X = O

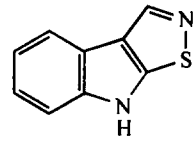
7, X = H, H; Y = S
8, X = Y = O



9, R = H
10, R = OCH_3



11



12

Fig. 1. Typical representatives of cruciferous phytoalexins

It was recently found, that besides their antimicrobial activity, brassinin and cyclobrassinin exhibit significant cancer chemopreventive activity.[21,22] Considering that indole phytoalexins are contained in the plant family Cruciferae and we take them from daily vegetables in a significant quantity,[23] it is quite important to investigate their biological activities.[24] Isolation from plants does not afford the sufficient quantities of indole phytoalexins for biological screening. These compounds can be also regarded as a leads for new biologically active compounds and therefore it is important to investigate the synthesis of indole phytoalexins and their analogs, to enable the study of their biological activity. The key intermediate in hitherto described syntheses of indole phytoalexins is 3-aminomethylindole. Thus brassinin (**1**) was synthesized by treatment of 3-aminomethylindole, prepared by the reduction of indole-3-carboxaldehyde oxime[25] with Devarda's alloy,[26] with CS_2 in the presence of pyridine and triethylamine and subsequent methylation of dithiocarbamate salt with CH_3I in 66 % yield.[11,12] Another three step synthesis of brassinin from indole in 58 % overall yield includes the conversion of gramine to 3-aminomethylindole and its subsequent reaction with CS_2 and CH_3I.[27] Brassinin was also synthesized in 72 % overall yield from indole-3-carboxaldehyde which was transformed to oxime, reduced to amine by H_2 on Raney nickel and amine treated with CS_2 and CH_3I in methanol, without isolation of

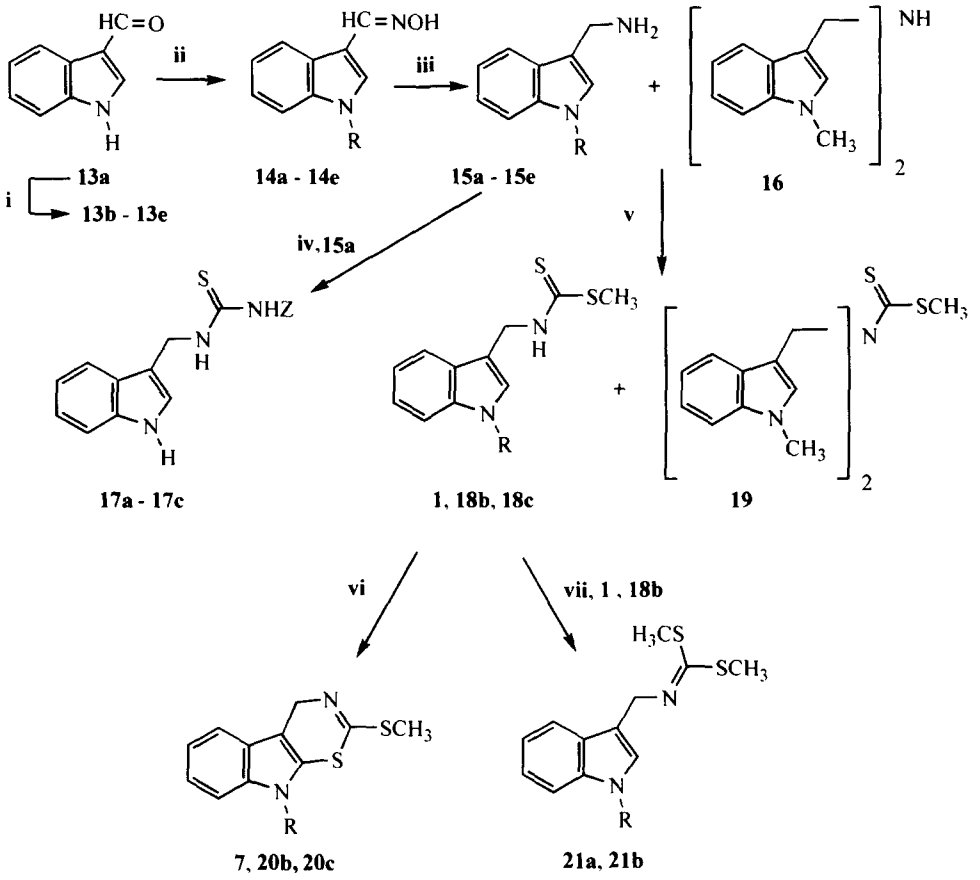
intermediates.[22] Methoxybrassinin (**2**) was obtained from indole-3-carboxaldehyde in 7 steps in 12 % overall yield, the last step being the reaction of 1-methoxy-3-aminomethylindole with CS_2 and CH_3I.[24] Analogously were prepared 2-methylbrassinin,[19,21] 4-methyl-, 5-methyl-, 7-methyl- and 5-chlorobrassinin,[21] 4-methoxy-, 4-iodo- and 4-nitrobrassinin,[27] N-methylbrassinin,[28] and 1-ethyl-2,3-dihydrobrassinin.[21] Cyclobrassinin can be prepared in 34-35 % yield by cyclization of brassinin with pyridinium bromide perbromide,[11,12] or NBS.[22] Brassitin was obtained in 8 % yield by oxidation of brassinin with H_2O_2.[10]

According to our knowledge, except of methoxybrassinin (**2**), no derivatives of brassinin and cyclobrassinin possessing a substituent on indole nitrogen or related compounds, having the SCH_3 group replaced by an alkoxy or amino group have been investigated so far. The aim of the present work was to synthesize the indole phytoalexins brassinin (**1**), cyclobrassinin (**7**), brassitin (**3**) and congeneric compounds *via* derivatives of 3-aminomethylindole and indol-3-ylmethyl isothiocyanate. We were also interested in antifungal activity of the prepared compounds.

RESULTS AND DISCUSSION

As the indole-3-carboxaldehyde oxime can be advantageously prepared by treatment of indole-3-carboxaldehyde with hydroxylamine hydrochloride in 96 % yield,[25] its reduction represents an attractive route to 3-aminomethylindole. Following the aim of our study it was decided to prepare the parent compound (**15a**), 1-methyl (**15b**), 1-Boc (**15c**), 1-phenylsulfonyl (**15d**) and 1-tosyl (**15e**) derivatives (Scheme 1). Commercially available indole-3-carboxaldehyde (**13a**) was methylated , *t*-butoxycarbonylated and arylsufonylated under phase transfer catalysis conditions, to afford its 1-substituted derivatives in 85-95 % yield. Previously, the aldehydes **13b-13e** were prepared by various methods, using sodium hydroxide in H_2O/dioxane (**13c**, 67 %)[29] or under anhydrous conditions (**13d**, 86 %).[30] Aldehyde **13d** has been prepared by PTC method, using tetrabutylammonium hydrogen sulfate as a catalyst in 73 % yield.[31] Our method, using tetrabutylammonium bromide is simple, time saving and generally applicable for the preparation of aldehydes **13b-13e**. The corresponding oximes **14a-14e** were obtained in high yields under the standard conditions as a mixtures of Z- and E-isomers. The reduction of oxime **14a** by Devarda's alloy[26,28] afforded in our hands only 40-45 % yield of **15a**, compared to 99 % described.[26] Similar observation was already mentioned by Somei.[27] In the case of 1-substituted oximes **14b-14e** this method did not work at all, and starting compounds were recovered. Although 3-aminomethyl-1-methylindole hydrogen sulfate can be obtained in 88 % yield by Raney nickel catalyzed hydrogenation of **14b**,[32] we looked for a general method, not using gaseous hydrogen. Therefore we elaborated a simplified modification of the nickel boride catalyzed reduction with sodium borohydride, previously used for the reduction of the aliphatic oximes.[33] By this method the amine **15a** was obtained in 45-50 % yield, however the amines **15b-15e** were prepared in 66-82 % yield. The best results were obtained, when the solid sodium borohydride was added in one portion into the solution of corresponding oxime and $NiCl_2.6H_2O$ in methanol. The reaction was finished within 5 min. In the case of 1-methyl oxime **14b** the yield was only about 10 %, probably because of the complexation of basic indole nitrogen to nickel chloride. This problem was solved by

slight modification of procedure, in which to a solution of $NiCl_2.6H_2O$ in methanol was first added sodium borohydride to produce nickel boride, then oxime **14b** and again sodium borohydride, thus affording the amine **15b** in 82 % yield.



For **13-15, 18, 20** and **21**; R = H (**a**), CH_3 (**b**), t-Boc (**c**), $PhSO_2$ (**d**), Tos (**e**). For **17**; Z = cyclo-C_6H_{11} (**a**), 4-CH_3-C_6H_4 (**b**), 4-Cl-C_6H_4-CO (**c**). *Reagents and conditions*: **i**, RX, 30 % NaOH/benzene, n-Bu_4NBr, rt, 85-95 %; RX = CH_3I (**b**), $(t\text{-Boc})_2O$ (**c**), $PhSO_2Cl$ (**d**), TosCl (**e**); **ii**, $H_2NOH.HCl$, Na_2CO_3, H_2O/ethanol, 50-100 °C, 81-98 %; **iii**, $NiCl_2.6H_2O$, $NaBH_4$, CH_3OH, rt, 45-82 %; **iv**, Z-NCS, CH_2Cl_2, rt, 80-95 %; **v**, for **1** and **18c**, CS_2, CH_3I, Et_3N, pyridine, 0-3 °C, 66-68 %; for **18b**, CS_2, CH_3I, CH_2Cl_2, rt, 53 %; **vi**, NBS, CH_2Cl_2, Et_3N, 30 °C, 31-61 %; **vii**, CH_3I, K_2CO_3, acetone, or CH_3I, LiH, DMF, rt, 40-56 %.

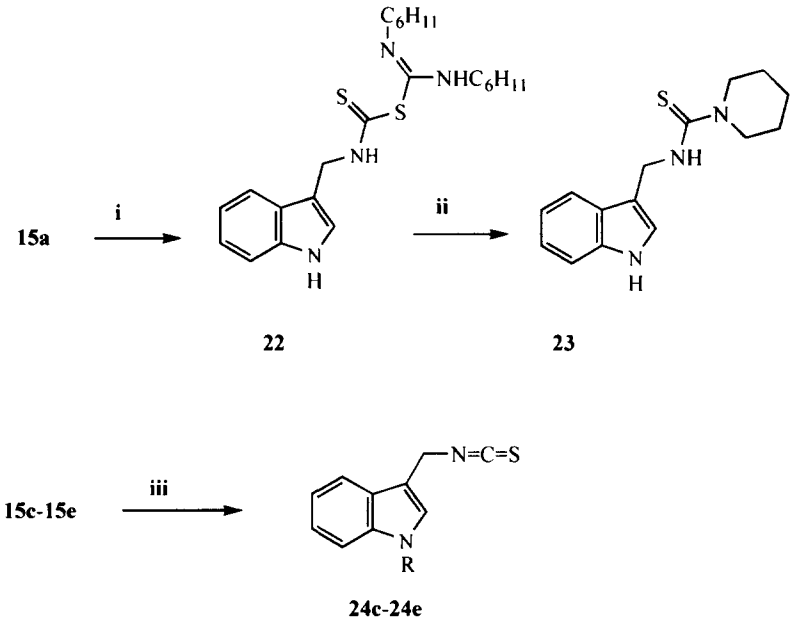
Scheme 1

Except of 3-aminomethylindole (**15a**), 1-substituted derivatives **15b-15e** are stable only in solution. After evaporation of the solvent, if not immediately redissolved, they decompose within a minute to unidentified material. Therefore no reasonable spectra of these compounds could be recorded and they were used as a crude

products immediately after isolation and quick weighing. With the aim to prepare the compounds related to brassinin, having the SCH_3 group replaced by an amino group, the amine **15a** was treated with selected isothiocyanates in dichloromethane, to yield thiourea derivatives **17a-17c** in 80-95 % yield (Scheme 1). Treatment of amines **15a-15c** with CS_2 and CH_3I in dichloromethane in the presence of triethylamine (**15b**), or under previously described conditions[11,12] (**15a**, **15c**), afforded brassinin (**1**) and its 1-substituted derivatives **18b** and **18c** in 53-68 % yield. With amines **15d** and **15e** their low reactivity toward carbon disulfide and rapid decomposition under the above conditions did not allow to prepare corresponding derivatives of brassinin. We have observed, that if during the preparation of **15b** the sodium borohydride was added portionwise within 10 min, or if the basic reaction mixture was left to stand at least 10 min before the work up, a substantial quantity of bis(1-methylindol-3-ylmethyl)amine (**16**) was produced and subsequent reaction with CS_2 and CH_3I resulted in the formation of a mixture of **18b** and N,N-bis(1-methylindol-3-ylmethyl)dithiocarbamate (**19**). The small quantity (up to 8 %) of **19** was always produced during the preparation of **18b**, probably because of the instability of **15b** in basic reaction mixture. Analogous 4-iodo and 4-nitroderivatives were previously obtained by Somei.[27] Treatment of **1**, **18b** and **18c** with NBS afforded the cyclobrassinin (**7**), and its 1-substituted derivatives **20b** and **20c**. Simple methylation of **1** and **18b** by CH_3I in acetone in the presence of K_2CO_3 or in DMF in the presence of LiH led to derivatives **21a** and **21b**, representing an analogs of methoxybrassenin A (**5**).

Our attention was next turned to the preparation of indol-3-ylmethyl isothiocyanate, a postulated biosynthetic intermediate of indole phytoalexins.[18,19] Its reactions should not only allow a biomimetic approach to indole phytoalexins, but should also enable the preparation of congeneric indoles, not available directly from amine **15a**. It is supposed, that this compound is formed during the enzyme myrosinase catalyzed hydrolysis of indole glucosinolate glucobrassicin at neutral pH as an unstable intermediate undergoing degradation to indole-3-carbinol and thiocyanate ion and thus contributing to anticarcinogenicity of brassica vegetables.[23] Although similar compounds, namely 3-indolyl isothiocyanate[34] and 2-(2-indolyl)ethyl isothiocyanate[35,36] are already known, indol-3-ylmethyl isothiocyanate has never been prepared, isolated or detected directly, whereas its more stable 1-methoxy analogue was detected by mass spectrometry as an indole glucosinolate neoglucobrassicin break down product.[37] To prepare indol-3-ylmethyl isothiocyanate, we have studied the reaction of amine **15a** with thiophosgene, representing a general method for preparation of isothiocyanates.[38] In a two-phase system , e.g. dichloromethane/water in the presence of K_2CO_3 or $CaCO_3$ we have only obtained a low yield of an amorphous red powder exhibiting in its IR spectrum the N=C=S absorption band at 1987 cm^{-1}, but in ^1H NMR spectrum only a broad signals were present, indicating its polymeric nature. It was supposed that in non-aqueous media the indole-3-ylmethyl isothiocyanate could be stable enough, to be isolated. Consequently, amine **15a** was treated with N,N'-diimidazolyl thione in anhydrous CCl_4,[39] or with CS_2 in anhydrous diethyl ether in the presence of dicyclohexylcarbodiimide.[40] Even these very gentle methods for preparation of isothiocyanates failed to afford stable indol-3-ylmethyl isothiocyanate. The later method resulted in the isolation of 71 % yield of white crystalline precipitate (m.p. 110-113 °C, decomp.) showing in the IR spectrum an intensive N=C=S absorption band at 2047 cm^{-1} and in the ^1H NMR spectrum, three signals were present in the CH_2 region at 4.66, 4.86 and 5.02 ppm. We suggest the structure of dicyclohexylisothiourea derivative (**22**, Scheme 2) for this

compound which can be present in two forms as a result of hindered rotation around N—C(S) bond as is also observed with brassinin.[12] All attempts to purify **22** by crystallisation or chromatography failed, because of its instability in solution, however the microanalytical and spectral data are consistent with the isothiourea structure. Compound **22** is unstable in solution and probably decomposes to N,N'-dicyclohexylthiourea and desired isothiocyanate, producing the NCS band in the IR spectrum and the third CH_2 signal in the ^1H NMR spectrum. This assumption was confirmed by treatment of **22** with piperidine resulting in the formation of the



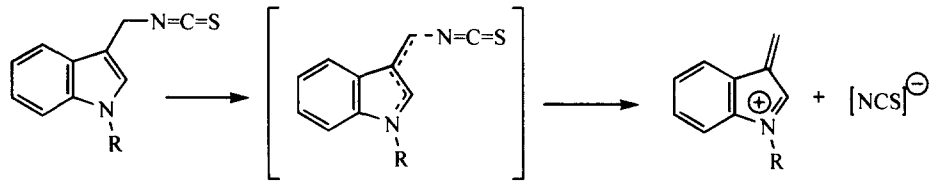
For **15** and **24**; R = t-Boc (**c**), $PhSO_2$ (**d**), Tos (**e**). *Reagents and conditions*: **i**, CS_2, DCC, diethylether -5 - 10° C to rt, 71 %; **ii**, piperidine, $CHCl_3$, rt, 28 %; **iii**, $CSCl_2$, $CaCO_3$, CH_2Cl_2, rt, 21-63 %.

Scheme 2

corresponding thiourea derivative **23** and N,N'-dicyclohexylthiourea. An equally unsuccessful attempt to prepare 1-methylindol-3-ylmethyl isothiocyanate, where no product could be isolated, indicates that the instability of indol-3-ylmethyl isothiocyanate can be explained by the activating effect of the indole nitrogen lone pair into 3-position resulting in the extrusion of the $[SCN]^-$ ion as suggested previously.[22] Consequently, the protection of indole nitrogen with an electron accepting group should increase the stability of indol-3-ylmethyl isothiocyanate. To support this hypothesis, we have performed the quantum chemistry calculations, using the AM1 method,[41] with the aim to assess a reaction pathway of $[SCN]^-$ splitting, with respect to the nature of protecting group. To achieve this goal, a series of model structures (**I-V**) was selected, in which the deprotonated species (**I**), and the methyl (**II**), hydrogen (**III**), methoxy (**IV**), and methoxycarbonyl (**V**) group is present as a substituent on indole nitrogen of indol-3-ylmethyl isothiocyanate. For all structures **I-V**, the

transition state for the proposed splitting reaction (Scheme 3), was found. The transition state structures were confirmed by calculation of vibrational frequencies and the corresponding IRC calculations. The comparison of the obtained activation enthalpies (Table 1) revealed, that the activation energy needed for the cleavage of the bond between CH_2 and NCS group increases with the increasing electron accepting nature of the substituent on indole nitrogen.

These predictions were confirmed by the stability of 1-Boc and 1-arylsulfonyl derivatives **24c-24e** obtained in 21-63 % yield by treatment of amines **15c-15e** with thiophosgene in CH_2Cl_2/H_2O in the presence of



I-V **TS**

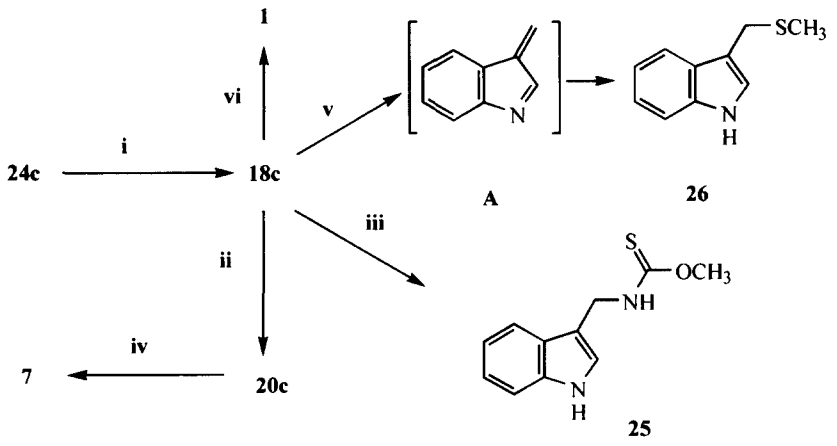
Scheme 3

Table 1. Activation Energies of Transition States

R	n	CH_3	H	OCH_3	$COOCH_3$
$\Delta H^{\#}$(kcal.mol^{-1})	25.64	40.42	58.49	59.92	65.93

$CaCO_3$ (Scheme 2). As anticipated, isothiocyanates **24c-24e** are stable solids, which after crystallisation can be stored at 0° C for several weeks without decomposition. With respect to the highest yield of isothiocyanate **24c** and expected easier removing of the Boc group, compared to arylsulphonyl group, compound **24c** was used as a biomimetic intermediate[42] for the synthesis of indole phytoalexins brassinin (**1**) and cyclobrassinin (**7**, Scheme 4). Nucleophilic addition of CH_3SNa to isothiocyanate **24c** in methanol afforded 97 % yield of protected brassinin (**18c**), which cyclized to cyclobrassinin analogue **20c** in 31 % yield by treatment with NBS.[22]

Attempted deprotection of **18c** and **20c** in acidic media, e.g. by trifluoroacetic acid,[43] or on silica gel surface[44] resulted in decomposition of starting compounds. Removing of the Boc group from indole derivatives can be also achieved by a 30 % solution of CH_3ONa in THF/CH_3OH.[45] In the case of **18c**, sodium methoxide removed the protecting group, but simultaneously replaced the SCH_3 by OCH_3 and corresponding monothiocarbamate **25** was formed. The less reactive SCH_3 group in **20c** is not replaced and cyclobrassinin (**7**) is obtained in 89 % yield. To achieve the deprotection of **18c**, we decided to use CH_3SNa, which however is a weaker base, compared to CH_3ONa. To enhance the reactivity of sodium methane thiolate, the reaction was performed in the presence of 15-crown-5-ether. The reaction proceeded well, however after about 30 min the reaction stopped and after one hour a new compound (**26**), was formed in the reaction mixture as the major product. It was suggested that S-methyl-O-(t-butyl)monothiocarbonate, formed after the removal of protecting



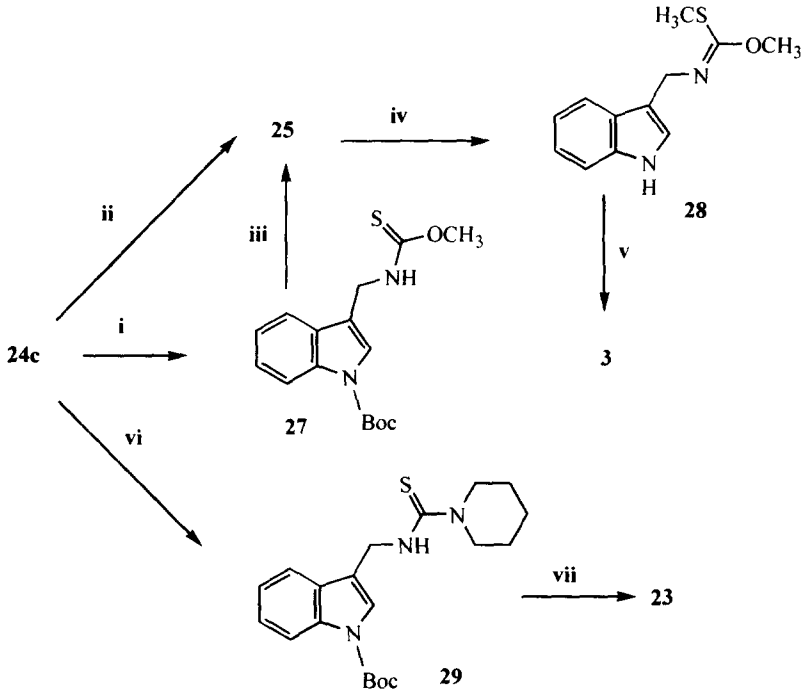
Reagents and conditions: **i**, CH_3SNa, CH_3OH, rt, 97 %; **ii**, NBS, Et_3N, CH_2Cl_2, 30 °C, 31 %; **iii**, CH_3ONa, 32 eq., CH_3OH, 71 %; **iv**, CH_3ONa, CH_3OH, rt, 89 %; **v**, CH_3SNa, 15-crown-5, CH_3CN, rt,70 min, 81 %; **vi**, CH_3SNa, 15-crown-5, piperidine, CH_3CN, rt, 85 min, 92 %.

Scheme 4

group by CH_3SNa was acting as a butoxycarbonylating agent and therefore under the used reaction conditions the reverse reaction could occur. On the other hand, the brassinin anion formed after deprotection could be stabilized by cleavage of the methyldithiocarbamate anion with the formation of indolenine intermediate (**A**), which can be subsequently intercepted by CH_3S^- ion resulting in the formation of **26**. Complete conversion of starting compound to brassinin was achieved by adding the piperidine or diphenylamine to the reaction mixture. However, when the reaction time is longer than 85 min, compound **26** was only slowly formed, and after several hours, no brassinin (**7**) was detected by t.l.c. Under optimized conditions (see experimental) the deprotection afforded 92 % yield of **1**.

To synthesize brassitin (**3**) from isothiocyanate **24c**, an oxygen atom had to be introduced into indole side chain. It is possibile to oxidize brassinin with H_2O_2 in methanol, however this method affords brassitin only in 8 % yield.[10] Therefore compound **27** (Scheme 5) was prepared by the reaction of **24c** with 11 equivalents of CH_3ONa. If 32 equivalents of CH_3ONa are used, the nucleophilic addition of sodium methoxide to NCS group is accompanied by simultaneous deprotection, and compound **25** was obtained in 51 % yield. Reaction of **27** with 6.5 equivalents of CH_3ONa proceeded smoothly with the formation of **25** in 81 % yield. Monothiocarbamates **25** and **27** exhibit in their ^1H NMR spectra the signals of minor rotamers, resulting from hindered rotation around the N—C(S) bond. An analogous situation has been previously observed with phytoalexin brassinin[12] and other monothiocarbamates.[46] Attempted rearrangement of monothiocarbamate **25** to brassitin (**3**) by treatment with boron trifluoride etherate,[47] or sulfuric acid in chloroform[48] led to an intractable mixture of decomposition products. Anyway, the monothiocarbamate **25** appeared to be the useful intermediate

in the synthesis of brassitin (**3**). Compound **28**, obtained by methylation of **25**, can be selectively hydrolyzed by diluted hydrochloric acid (1:1) in THF, preserving the methythio and hydrolyzing the methoxy group, and thus affording the desired brassitin (**3**) in 87 % yield. Compound **28** can be obtained in a "one pot" reaction from isothiocyanate **24c**, using the sodium methoxide in methanol not only as a nucleophile, but also as a deprotecting agent and as the base, needed for methylation. This approach afforded brassitin (**3**) from **24c** in 68 % overall yield. Whereas amine **15c** reacts readily with isothiocyanates to produce N,N'-disubstituted thiourea derivatives **17a-17c**, the preparation of corresponding trisubstituted thiourea derivatives requires the use of indol-3-ylmethyl isothiocyanate and secondary amine. Thus isothiocyanate **24c** was treated with piperidine to afford protected thiourea derivative **29**, which after deprotection with CH_3ONa afforded compound **23**, identical with the product of the reaction of **22** with piperidine.



Reagents and conditions: **i**, CH_3ONa, 11 eq, CH_3OH, rt, 58 %; **ii**, CH_3ONa, 32 eq, CH_3OH, rt, 51 %; **iii**, CH_3ONa, 6.5 eq, CH_3OH, rt, 81 %; **iv**, CH_3ONa, 3 eq, CH_3OH , CH_3I, rt, 89 %; **v**, HCl (1:1), THF, rt, 87 %; **vi**, piperidine, CH_2Cl_2, rt, 57 %; **vii**, CH_3ONa, 32.6 eq, CH_3OH, rt, 62 %.

Scheme 5

Antifungal activity of the prepared compounds was examined, using the fungus *Bipolaris leersiae* by TLC bioassay . The compounds, showing a distinct antifungal spots were tested quantitatively and compared with brassinin (**1**). None of the investigated compounds was found to have the antifungal activity higher than brassinin, which completely inhibited the conidial germination of the fungus at concentration of 0.1 $mmol.l^{-1}$.

1-Methyl brassinin (**18b**) exhibited the same activity as brassinin and of the other derivatives, the thiourea **17a** completely inhibited the germination at the concentration of 1 mmol.l^{-1} and isothiocyanate **24c** at 0.25 mmol.l^{-1}. None of the other compounds showed any significant antifungal activity.

CONCLUSION

An approach to indole phytoalexins brassinin, cyclobrassinin, brassitin and related compounds from 3-aminomethylindole has been elaborated for the preparation of its 1-methyl, 1-Boc, 1-phenylsulfonyl and 1-tosyl derivatives. The derivatives possessing an electron accepting group in 1-position afforded, by treatment with thiophosgene, the stable derivatives of indol-3-ylmethyl isothiocyanate. 1-Boc analogue was successfully used as a biomimetic intermediate in the synthesis of indole phytoalexins. None of the new derivatives were found to exhibit a higher activity against the fungus *Bipolaris leersiae*, than phytoalexin brassinin.

EXPERIMENTAL

Melting points were determined on a Koffler hot-stage apparatus and are uncorrected. Infrared spectra were recorded on an IR-75 (Zeiss, Jena) spectrometer in chloroform in the region 400-4000 cm^{-1}; the wavenumbers are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were measured on Tesla BS 487 (80 MHz, for ^{1}H), Tesla BS 567 (25.15 MHz for ^{13}C, compound **24c**) and Bruker Avance DRX-500 spectrometer (125.16 MHz for ^{13}C, compounds **18b, 20b, 28**) in deuteriochloroform solutions unless otherwise stated. Chemical shifts (δ) are reported in ppm downfield from TMS. The mass spectra were recorded on a JMS-100D spectrometer (Jeol) at ionization energy 70 eV. Microanalyses were performed with a Perkin-Elmer, Model 2400 analyzer. The reaction course was monitored by TLC on Silufol plates (Kavalier®, Czech Republic). The preparative column chromatography (flash chromatography) was performed over the Kieselgel Merck Typ 9385, 230-400 mesh. Starting indole-3-carboxaldehyde oxime (**14a**)[25] and 1-methylindole-3-carboxaldehyde oxime (**14b**)[32] were prepared according to the literature procedures. For the TLC bioassay, the samples were spotted on silica gel TLC sheets (Kieselgel 60 F254, Merck) and developed with diethyl ether. The TLC sheets were sprayed with a conidial suspension of the fungus *Bipolaris leersiae* in potato-glucose medium, and incubated for 48 h at 25 °C in a humid case. Compounds with antifungal activity appeared as white spots against a black background formed by conidia and mycelium of the fungus. For the quantitative screening of antifungal activity, the conidia obtained from a Petri dish of *Bipolaris leersiae*, seeded and incubated for 13 days at 25 °C before use, were suspended in a mixture of water (100 ml), 1/15 M KH_2PO_4 (100 ml), and potato dextrose broth (2 ml). Under stirring, 1 ml each of the suspension was taken into each 10 ml vial. Each 10 μl of the acetone solution of samples was added to each vial. The vials were capped and incubated for 20 h at 25 °C, and the germination of conidia was examined under a microscope.

1-Substituted indole-3-carboxaldehydes ***13b-13e.*** To a vigorously stirred solution of indole-3-

carboxaldehyde (2g, 13.8 mmol) in benzene (50 ml) was added 30 % solution of sodium hydroxide (50 ml), tetrabutylammonium bromide (440 mg, 1.38 mmol) and methyl iodide, di-tert-butyldicarbonate, benzenesulfonylchloride or 4-toluenesulfonylchloride (14.49 mmol) and the stirring was continued at room temperature for 20 min (**13b**), 5 min (**13c**), 30 min (**13d** and **13c**). The benzene layer was separated and water layer extracted with benzene (20 ml). In the case of **13b** the collected benzene extract was washed with 5 % solution of potassium hydroxide (50 ml). After drying with anhydrous Na_2SO_4 and evaporation of benzene, the corresponding aldehyde was obtained by crystallization from the appropriate solvent. 1-Methylindol-3-carboxaldehyde (**13b**): yield 95 %, m.p. 68-70 °C (hexane/light petroleun), lit.[32] 69-70 °C; 1-(t-butoxycarbonyl)indol-3-carboxaldehyde (**13c**): yield 90 %, m.p. 123-125 °C (benzene/light petroleum), lit.[49] 124 °C, lit.[29], 124.5-125.5 °C; 1-phenylsulfonylindol-3-carboxaldehyde (**13d**): yield 85 %, m.p. 156-158 °C (hexane), lit.[30] 157.5-158.5 °C; 1-(4-toluenesulfonyl)indol-3-carboxaldehyde (**13e**): yield 90 %, m.p. 147-149 °C (hexane), lit.[50] 148-150 °C.

*1-Substituted indole-3-carboxaldehyde oximes **14c-14e**.* To a stirred solution of corresponding aldehyde (8.12 mmol) in ethanol (15 ml) was added a solution of hydroxylammonium chloride (884 mg, 12.72 mmol) and sodium carbonate (624 mg, 5.88 mmol) in water (2.4 ml), and the mixture was stirrer with heating for 5 min at 50 °C (**14c**), refluxed for 10 min (**14d**) or heated at 90 °C for 20 min (**14e**). After evaporation of ethanol and addition of water (20 ml), the oxime **14c** was extracted with diethyl ether (40 ml) and after drying with anhydrous Na_2SO_4 the solvent evaporated, whereas separated precipitates of **14d** and **14e** were filtered with suction, dried and crystallized from ethanol/water. 1-(tert-Butoxycarbonyl)indol-3-carboxaldehyde oxime (**14c**): Yield 98 %, colorless oil; IR: 1625 (C=N), 1730 (C=O), 3587 (O-H); ^{1}H NMR (acetone-d_6): 1.69 s and 1.71 s, 9H $C(CH_3)_3$; 7.38 m, 2H and 8.20 m, 2H, $-C_6H_4$; 7.86 s and 7.96 s, (1:2), 1H, CH=N; 8.35 s and 8.67 s (2:1), 1H, H-2; 10.30 s, 1H, OH. Anal. calcd. for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76 ; found: C, 64.51; H, 6.32; N, 10.59. 1-Phenylsulfonylindol-3-carboxaldehyde oxime (**14d**): Yield 90 %, mp 198-200 °C; IR: 1121 and 1366 (SO_2), 1622 (C=N), 3587 (O-H); ^{1}H NMR (DMSO-d_6): 7.43 m, 5H and 7.93 m 5H, C_6H_5, $-C_6H_4$- and CH=N; 8.27 s and 8.71 s (10:1), 1H, H-2; 10.79, s,1H, OH. Anal. calcd. for $C_{15}H_{12}N_2O_3S$: C, 59.99; H, 4.03; N, 9.33; found: C, 59.78; H, 4.19; N, 9.50. 1-(4-Toluenesulfonyl)indol-3-carboxaldehyde oxime (**14e**): Yield 81 %, mp 179-181 °C; IR: 1127 and 1373 (SO_2), 1593 (C=N), 3587 (O-H). ^{1}H NMR ($CDCl_3$-DMSO-d_6, 12:1): 2.33 s, 3H, CH_3; 7.29 m, 4H and 7.90 m, 5H, 4-CH_3-C_6H_4, $-C_6H_4$- and CH=N; 8.28 s and 8,73 s (2.8:1), 1H, H-2; 10.56 s, 1H, OH. Anal. calcd. for $C_{16}H_{14}N_2O_3S$: C, 61.13; H, 4.49; N, 8.91; found: C, 61.02; H, 4.63, N, 9.12.

*1-Substitutedindol-3-ylmethyl amines **15a-15e**.* To a solution of $NiCl_2.6H_2O$ (740 mg, 3.1 mmol) in methanol (50 ml) was added corresponding oxime **14a-14e** (3.1 mmol) and $NaBH_4$ (760 mg, 20 mmol) was added in one portion with stirring. In the case of **15b**, to a solution of $NiCl_2.6H_2O$ was first added $NaBH_4$ (3.1 mmol), then oxime and finally 16.9 mmol of $NaBH_4$. After 5 min the black precipitate was filtered off, filtrate concentrated in vacuum to approx . 1/3 of its original volume and poured into 200 ml of water containing 8 ml of 24 % NH_4OH. After extraction with ethyl acetate (1x150 and 2x80 ml), drying the extract with anhydrous

Na_2SO_4 and evaporation of the solvent, the crude amines **15a-15e** were obtained as a viscous, slightly yellow oils. Amines **15b** (82%), **15c** (75%), **15d** (71%) and **15e** (67%) could not be purified because of their high instability and were used for further reaction as a crude products immediately after isolation and quick weighing. 3-Aminomethylindole (**15a**) was obtained as a white crystals after flash chromatography on 15g of silica gel , eluent[28] dichloromethane/methanol/24 % NH_4OH (80:20:1). Yield 45 %, mp 102-104 °C (CH_2Cl_2/hexane), lit.[26] 103-105 °C (benzene).

*N-Substituted-N'-(indol-3-ylmethyl)thioureas **17a-17c***. A solution of amine **15a** (292 mg, 2 mmol) in dichloromethane (30 ml) was added to a solution of cyclohexyl, 4-methylphenyl or 4-chlorobenzoyl isothiocyanate (2.2 mmol) in dichloromethane (10 ml). After standing for 24 h (**17a**), 1.5 h (**17b**) or 0.5 h (**17c**) at room temperature, 40 ml of hexane (**15a**, **15b**), or light petroleum (**15c**) was added and after standing for 1h at 0 °C, the separated crystalline precipitate was filtered off. N-Cyclohexyl-N'-(indol-3-ylmethyl)thiourea (**17a**): Yield 83 %, mp 142-144 °C (CH_2Cl_2/hexane); IR: 1525 (NHCS), 3425 and 3485 (N-H); ^{1}H NMR ($CDCl_3$-DMSO-d_6, 11:1): 1.05-3.10 m, 10 H, $(CH_2)_5$; 4.15 m, 1H, CH; 4.87 d, J=3 Hz, 2H, CH_2; 6.70 br. s, 1H, NH; 6.92 br. s, 1H, NH; 7.25 m, 3H, 7.43 m, 1H and 7.69 m, 1H, 3-substituted indole; 9.78 br. s, 1H, NH. Anal calcd. for $C_{16}H_{21}N_3S$: C, 66.86; H, 7.36; N, 14.62; found: C,66.71; H, 7.50; N, 14.53. N-(4-methylphenyl)-N'-(indol-3-ylmethyl)thiourea (**17b**): Yield 95 %, mp 133-135 °C (CH_2Cl_2/hexane); IR: 1507 (NHCS), 3390 and 3483 (N-H); ^{1}H NMR (acetone-d_6): 2.25 s, 3H, CH_3; 5.01 dd, J_1=5 Hz, J_2=1 Hz, 2H, CH_2; 7.13 m, 6H, 7.36 m, 2H and 7.73 m, 1H, 3-substituted indole and -C_6H_4-; 8.75 br. s, 1H, NH; 9.65 br. s, 1H, NH; 11.00 br. s, 1H, NH. Anal. calcd. for $C_{17}H_{21}N_3S$: C, 69.12; H, 5.80; N, 14.23; found: C, 68.92; H, 5.67; N, 14.44. N-(4-Chlorobenzoyl)-N'-(indol-3-ylmethyl)thiourea (**17c**): Yield 81 %, mp 113-115 (CH_2Cl_2/light petroleum, 0 °C); IR: 1505 (NHCS), 3440 and 3483 (N-H); ^{1}H NMR : 5.03 d, J=4 Hz, 2H, CH_2; 7.31 m, 6H and 7.72 m, 3H, 3-substituted indole and -C_6H_4-; 8.33 br. s, 1H, NH; 9.02 br. s, 1H, NH; 10.78 br. s, 1H, NH. Anal. calcd. for $C_{17}H_{14}ClN_3OS$: C, 59.38; H, 4.10; N, 12.22; found: C, 59.11; H, 4,25; N, 11.98.

*1-Methylbrassinin **18b***. To a stirred solution of crude, freshly prepared amine **15b** (760 mg, 4.74 mmol) in dichloromethane (50 ml) was added triethylamine (0.48 g, 0.66 ml, 4.74 mmol) and carbon disulfide (0.72 g, 0.44 ml, 9.48 mmol). After stirring for 5 min at room temperature, methyl iodide (0.8 g, 0.35 ml, 5.68 mmol) was added and stirring was continued for additional 5 min. The solvent was evaporated and the residue chromatographed on 80 g of silica gel, eluent benzene/acetone (19:1), to yield 632 mg (53 %) of **18b** and 75 mg (8%) of **19**. 1-Methylbrassinin (**18b**): Mp 112-114 °C (benzene/hexane); IR: 1463 (NHCS), 3387 (N-H); ^{1}H NMR (acetone-d_6): 2.58 s, 3H, SCH_3; 3.80 s, 3H, NCH_3; 5.08 d, J=5 Hz, 2H, CH_2; 7.15 m, 2H, 7.36 m, 1H, 7.68 m, 1H, and 7.30 s, 1H, 1,3-disubstituted indole. ^{13}C NMR (acetone-d_6): 17.86 (SCH_3), 32.77 (NCH_3), 43.01 (CH_2), 110.28, 110.70 (q), 119.83, 119.91, 122.45, 128.35 (q), 129.81, 138.06 (q), 198.43 (C=S). Anal. calcd. for $C_{12}H_{14}N_2S_2$: C, 57.56; H, 5.64; N, 11.19; found: C, 57.32; H, 5.79; N, 11.35. N,N-bis(1-Methylindol-3-ylmethyl)dithiocarbamate (**19**): Mp 149-150 °C (acetone/hexane); IR: 1460 (N-C=S); ^{1}H NMR: 2.73 s, 3H, SCH_3; 3.80 s, 6H, 2 x NCH_3, 5.14 br.s, 2H and 5.45 br. s, 2H, CH_2NCH_2, 7.24 m, 8H and 7.65 m, 2H, 2 x 1,3-

disubstituted indole. Anal. calcd. for $C_{22}H_{23}N_3S_2$: C, 67.14; H, 5.89; N, 10.68; found: C, 67.28; H, 5.63; N, 10.81.

1-(t-Butoxycarbonyl)brassinin ***18c***. Method A: Using the literature procedure,[12] compound **18c** was obtained from the freshly prepared amine **15c** in 68 % yield. Method B: To a stirred solution of isothiocyanate **24c** (0.15g, 0.52 mmol) in methanol (10 ml) was added within 10 min a solution of sodium methane thiolate (0.037 g, 0.53 mmol) in methanol (5 ml). After additional 5 minutes, the reaction mixture was poured into 150 ml of water and product extracted with three 20 ml portions of chloroform. After drying of the extract with anhydrous Na_2SO_4 and evaporation of the solvent, the crystallization of the residue afforded 0.17 g (97 %) of **18c**. Mp 103-105 °C (hexane); IR: 1467 (NHCS); 1727 (C=O), 3380 (N-H); ^{1}H NMR: 1.68 s, 9H, $(CH_3)_3$, 2.60 s, 3H, SCH_3, 5.12 d, J=5 Hz, 2H, CH_2; 7.31 m, 2H, 7.70 m, 1H, 7.73 s, 1H and 8.15 m, 1H, 1,3-disubstituted indole; 9.20 br. s, 1H, NH. Anal. calcd. for $C_{15}H_{16}N_2O_2S_2$: C, 57.11; H, 5.99; N, 8.33; found: C, 57.02; H, 6.18; N, 8.13.

N-Substituted derivatives of cyclobrassinin ***20b, 20c***. Using the literature procedure,[22] compounds **20b** and **20c** were prepared from brassinin analogues **18b** and **18c**. N-Methylcyclobrassinin (**20b**): Yield 61 %, mp 97-99 °C (CH_2Cl_2/light petroleum); IR: 1600 (C=N); ^{1}H NMR (acetone-d_6): 2.55 s, 3H, SCH_3; 3.67 s, 3H, NCH_3; 5.03 s, 2H, CH_2; 7.15 m, 2H and 7.48 m, 2H -C_6H_4-; ^{13}C NMR (acetone-d_6): 15.26 (SCH_3), 30.30 (NCH_3), 49.57 (CH_2), 103.12 (q), 109.93, 117.96, 120.46, 122.24, 125.53 (q), 125.86 (q), 139.17 (q), 151.97 (C=N); EIMS, m/z (%): 248 (M^+, 21), 175 (100), 174 (37), 142 (16), 130 (18), 115 (8). Anal. calcd. for $C_{12}H_{12}N_2S_2$: C, 58.03; H, 4.87; N, 11.28; found: C, 57.89; H, 4.98; N, 11.02. N-(t-Butoxycarbonyl)cyclobrassinin (**20c**): Yield 31 %, mp 97-99 °C (light petroleum); IR: 1600 (C=N), 1720 (C=O); ^{1}H NMR (acetone-d_6): 1.71 s, 9H, $(CH_3)_3$; 2.52 s, 1H, SCH_3; 5.04 s, 2H, CH_2; 7.30 m, 2H, 7.53 m, 1H and 8.11 m, 1H, -C_6H_4-. Anal. calcd. for $C_{16}H_{18}N_2O_2S_2$: C, 57.46; H, 5.42; N, H, 8.38; found: C, 57.32; H, 5.60; N, 8.25.

Analogs of methoxybrassenin A ***21a, 21b***. Brassenin A (**21a**): To a suspension of LiH (4 mg, 0.5 mmol) in DMF (4 ml) was added brassinin (**1**, 100 mg, 0.42 mmol). After stirring for 30 min at room temperature, the methyl iodide (119 mg, 0.052 ml, 0.84 mmol) was added and the stirring was continued for 1 h. Reaction mixture was under efficient stirring slowly poured into 40 ml of cold water and separated precipitate was filtered with suction. Yield 43 mg (40%), mp 136-138 °C (CH_2Cl_2/hexane); IR: 1562 (N=C-S), 3487 (N-H); ^{1}H NMR: 2.40 s and 2.60 s (1:1), 6H, 2 x SCH_3; 4.80 s, 2H, CH_2; 7.13 m, 3H, 7.36 m, 1H and 7.67 m, 1H, 3-substituted indole; 9.48 br. s, 1H, NH. Anal. calcd. for $C_{12}H_{14}N_2S_2$: C, 57.56; H, 5.64; N, 11.19; found: C, 57.69, H, 5.48; N, 11.07. N-Methylbrassenin A (**21b**): To a solution of 1-methylbrassinin (**18b**, 50 mg, 0.2 mmol) in dry acetone (4 ml) was added K_2CO_3 (31 mg, 0.22 mmol) and CH_3I (85 mg, 0.037 ml, 0.6 mmol) and the mixture was heated to 40-45 °C with stirring for 30 h. Insoluble material was filtered off, washed with acetone, filtrate evaporated and the residue chromatographed on 10 g of silica gel, eluent cyclohexane/acetone (2:1). Yield 30 mg (56 %), gum. IR: 1583 (C=N); ^{1}H NMR: 2.40 s and 2.59 s (1:1), 6H, 2 x SCH_3; 3.74 s, 3H,

NCH_3; 4.80 s, CH_2; 7.03 s, 1H, 7.23 m, 3H and 7.68 m, 1H, 1,3-disubstituted indole. Anal. calcd. for $C_{13}H_{16}N_2S_2$: C, 59.05; H, 6.10; N, 10.60; found: C, 59.12; H, 5.99; N, 10.48.

N,N'-Dicyclohexyl-S-[N-(indol-3-ylmethyl)thiocarbamoyl]isothiourea ***22***. Dry CS_2 (1.512 g, 1.2 ml, 40 mmol) was added with stirring and cooling (-10 °C) to a solution of DCC (412 mg, 2 mmol) in 35 ml of anhydrous diethylether. Solid 3aminomethylindole (**15a**, 292 mg, 2 mmol) was added in one portion, and stirring was continued with cooling for another 20 min. Then the reaction mixture was left to warm up to room temperatute and was set asside at room temperature for 3 h. Hexane (30 ml) was added and the mixture was left to stand at 0 °C overnight. Separated white crystalline precipitate was filtered off and washed with hexane. Yield 660 mg (71 %), mp 110-113 °C, decomp; IR: 1535 (NHCS), 1617 (C=N), 2047 br. (N=C=S), 3300, 3420 and 3483 (N-H); ^{1}H NMR (acetone-d_6): 1.00 - 1.91 m, 20 H, 2 x $(CH_2)_5$; 4.10 m, 2H, 2 x CH; 4.66 s, 4.86 s and 5.02 d, J = 1 Hz (4:2:1), 2H, CH_2, 6.59 br. s, 1H, NH; 7.15 m, 2H, 7.46 m, 2H and 7.70 m, 1H, 3-substituted indole. Anal. calcd. for $C_{23}H_{32}N_4S_2$: C, 64.44; H, 7.52; 13, 07; found: C, 64.90; H, 7.83; N, 13.49.

1-[N-(Indol-3-ylmethyl)thiocarbamoyl]piperidine ***23***. Method A: A solution of **22** (428 mg, 1 mmol) in chloroform (25 ml) was stirred at room temperature for 10 min. Then piperidine (129 mg, 1.15 ml, 1.5 mmol) was added and the mixture was stirred for 1 h at room temperature. After evaporation of solvent the residue was chromatographed on 40 g of silica gel, eluent cyclohexane/acetone (2:1) to yield N,N'-dicyclohexylthiourea (190 mg, 79 %) and 75 mg (28 %) of **23**, mp 128-130 °C (acetone/cyclohexane). Method B: To a solution of thiourea derivative **29** (150 mg, 0.4 mmol) in dry methanol (10 ml) was added sodium (300 mg, 23.05 mmol) in small pieces within 15 min with stirring without cooling and the mixture was stirred for additional 10 min. Then the mixture was poured into cold water (100 ml), the product extracted with chloroform (2 x 20 ml), extract dried with anhydrous Na_2SO_4 and solvent evaporated. Yield 68 mg (62 %), mp 126-128 °C (CH_2Cl_2/hexane); IR: 1500 (NHCS), 3440 and 3480 (N-H); ^{1}H NMR: 1.63 m, 6H, $(CH_2)_3$; 3.75 m, 4H, $N(CH_2)_2$; 5.01 d, J = 4 Hz, 2H, CH_2; 6.52 br. s, 1H, NH; 7.21 m, 3H, 7.40 m, 1H and 7.65 m, 1H, 3-substituted indole; 8.27 br. s, 1H, NH. Anal. calcd. for $C_{15}H_{19}N_3S$: C, 65.90; H, 7.00; N, 15.37; found: C, 65.81; H, 7.12; N, 15.23.

(1-Substituted indol-3-yl)methyl isothiocyanates ***24c-24e***. A solution of freshly prepared amine **15c-15e** (1.48 mmol) in CH_2Cl_2 (7.5 ml) was added dropwise during 5 min to a vigorously stirred mixture of $CSCl_2$ (189 mg, 1.48 mmol) and $CaCO_3$ (188 mg, 1.88 mmol) in 7.5 ml of CH_2Cl_2 and 13 ml of water. Stirring was continued for additional 5 min, the layers separated and water layer extracted with CH_2Cl_2 (5 ml). The dichloromethane solution was filtered on charcoal, the solvent evaporated and the residue chromatographed on 20 g of silica gel, eluent benzene. [1-(tert-Butoxycarbonyl)indol-3-yl]methyl isothiocyanate (**24c**): Yield 63 %, mp 66-68 °C (hexane, -10 °C); IR: 1720 (C=O), 2082 (br. intensive band, N=C=S); ^{1}H NMR: 1.68 s, 9H, $(CH_3)_3$; 4.55 d, J = 1 Hz, 2H, CH_2; 7.23 m, 4H and 7.93 m, 1H , 1,3-disubstituted indole; ^{13}C NMR: 28.26 $C(\underline{C}H_3)_3$, 40.84 CH_2, 84.25 C-O, 114.41 (q), 116.60, 118.70, 123.14, 124.30, 125.16, 128.37 (q), 134.00 (br. N=C=S), 135.80 (q), 149.42 (C=O); EIMS, m/z (%): 288 (M^+, 52), 232 (80), 215 (10), 174 (57), 130 (100), 102

(10), 57 (90). Anal calcd. for $C_{15}H_{16}N_2O_2S$: C, 62.47; H, 5.59; N, 9.71; found: C, 62.58; H, 5.41; N, 9.96. (1-Phenylsulfonylindol-3-yl)methyl isothiocyanate (**24d**): Yield 21 %, mp 125-127 °C (benzene/hexane); IR: 1117 and 1367 (SO_2), 2080 (br. N=C=S); ^{1}H NMR: 4.79 d, J = 1Hz, 2H, CH_2; 7.43 m, 6H and 7.90 m, 4H, C_6H_5- and 1,3-disubstituted indole. Anal. calcd. for $C_{16}H_{12}N_2O_2S_2$: C, 58.52; H, 3.68; N, 8.53; found: C, 58.30; H, 3.77; N, 8.71. [1-(4-Toluenesulfonyl)indol-3-yl]methyl isothiocyanate (**24e**): Yield 44 %, mp 145-147 °C ($CHCl_3$/light petroleum); IR: 1120 and 1369 (SO_2), 2080 (br. N=C=S); ^{1}H NMR: 2.34 s, 3H, CH_3; 4.77 d, J = 1Hz, 2H, CH_2; 7.13 - 8.10 m, 9H, -C_6H_4- and 1,3-disubstituted indole. Anal. calcd. for $C_{17}H_{14}N_2O_2S_2$: C, 59.63; H, 4.12; N, 8.18; found: C, 59.48; H, 4.27; N, 8.31.

O-Methyl N-(indol-3-yl)methyl monothiocarbamate ***25***. *Method A:* To a solution of 1-(*t*-butoxycarbonyl)brassinin (**18c**, 100 mg, 0.32 mmol) in dry methanol (10 ml) was added sodium (300 mg, 13.05 mmol) in small pieces within 15 min with stirring and ice cooling. The mixture was then poured into 100 ml of cold water, product extracted with chloroform (3 x 30 ml), the extract dried with anhydrous Na_2SO_4 and solvent evaporated. Yield 50 mg (71 %). *Method B:* To a stirred solution of isothiocyanate **24c** (390 mg, 1.35 mmol) in dry methanol (20 ml) was added sodium (1g, 43.5 mmol) in small pieces within 20 min. The mixture was poured into 200 ml of cold water and extracted with chloroform (3 x 30 ml), the extract dried with anhydrous Na_2SO_4 and solvent evaporated. The residue was chromatographed on 60 g of silica gel, eluent benzene/acetone (19:1), yielding after crystallization 197 mg (67 %) of **25**. *Method C:* Analogously to previous procedure the product **25** was obtained from **27** (160 mg, 0.5 mmol) and 75 mg (3.25 mmol) of sodium in 10 ml of dry methanol in 81 % yield. Mp 74-76 °C (CH_2Cl_2/hexane); IR: 1490 (NHCS), 3400 and 3486 (N-H); ^{1}H NMR: 3.98 s and 4.14 s (2:1), 3H, OCH_3; 4.58 d, J=2 Hz and 4.80 d, J=2 Hz (1:2), 2H, CH_2; 6.38 br. s and 6.73 br. s (2:1), 1H, NH; 7.20 m, 4H and 7.60 m, 1H, 3-substituted indole, 8.13, br. s, 1H, NH. Anal. calcd. for $C_{11}H_{12}N_2OS$: C, 59.97; H, 5.49; N, 12, 72; found: C, 59.82; H, 5.60; N, 12.89.

Removal of t-Boc protecting group from ***18c***. A stirred solution of isothiocyanate **24c** (100 mg, 0.346 mmol) in dry CH_3CN (15 ml) was treated with CH_3SNa (242 mg, 3.46 mmol). After 10 min, when the formation of **18c** was completed, 330 mg (1.5 mmol) of 15-crown-5 ether and 88 mg (1.04 mmol) of piperidine were added and stirring was continued for 85 min. The mixture was poured into 150 ml of water and extracted with diethyl ether (1x50 and 2x30 ml). After drying the extract with anhydrous Na_2SO_4 and evaporation of the solvent, the residue was chromatographed on 30 g of silica gel, eluent cyclohexane/acetone (2:1), yielding 75 mg (92 %) of brassinin (**1**), mp 131-133 °C (CH_2Cl_2/hexane), lit.[12] 132-133 °C.

3-Methylthioindole ***26***. Under the same conditions as in the previous case, but with the reaction time 10 h, compound **26** was isolated as the sole reaction product. Yield 50 mg (81 %), mp 85-87 °C (CH_2Cl_2/hexane), lit.[51], 87-88 °C.

Removal of t-Boc protecting group from ***20c***. To a stirred solution of cyclobrassinin analogue **20c** (80 mg, 0.239 mmol) in dry methanol (10 ml) was added in small pieces within 15 min 150 mg (6.5 mmol) of sodium. The mixture was poured into 100 ml of water, product extracted with chloroform (3x40 ml), extract dried with anhydrous Na_2SO_4 and the solvent evaporated. Yield 50 mg (89 %) of cyclobrassinin (**7**), mp 135-137 °C (CH_2Cl_2/hexane), lit.[12], 136-137 °C.

O-Methyl N-[1-(tert-butoxycarbonyl)indol-3-yl]methyl monothiocarbamate ***27***. To a stirred solution of isothiocyanate **24c** (144 mg, 0.5 mmol) in dry methanol (13 ml) was added sodium (125 mg, 5.5 mmol) in small pieces within 15 min. The obtained solution was poured into water (150 ml) and the product extracted with chloroform (3x20 ml), extract dried with $MgSO_4$ and solvent evaporated. Yield 130 mg (84 %), mp 76-78 °C (CH_2Cl_2/hexane). IR: 1490 (NHCS), 1720 (C=O), 3407 (N-H); ^{1}H NMR: 1.67 s, 9H, $(CH_3)_3$; 4.01 s and 4.14 s (2:1), 3H, OCH_3; 4.59 d, J=5 Hz and 4.89 d, J=5 Hz (1:2), 2H, CH_2; 6.40 br. s and 6.78 br.s (2:1), 1H, NH; 7.34 m, 2H, 7.60 m, 2H and 8.16 m, 1H, 1,3-disubstituted indole. Anal. calcd. for $C_{16}H_{20}N_2O_3S$: C, 59.98; H, 6.29; N, 8.74; found: C, 59.77; H, 6.45; N, 8.63.

O-Methyl-S-methyl-N-(indol-3-ylmethyl)iminomonothiocarbonate ***28***. To a stirred solution of isothiocyanate **24c** (288 mg, 1 mmol) in dry methanol (14 ml) was added sodium (738 mg, 32 mmol) in small pieces within 15 min without cooling and the mixture was stirred for additional 10 min. Then the reaction mixture was diluted with dry methanol (20 ml), methyl iodide (710 mg, 0.622ml, 5 mmol) was added and stirring at room temperature was continued for additional 15 min. After dilution with water (40 ml) the mixture was neutralized to pH~7 with 1M HCl and diluted with water to final volume about 250 ml. The product was extracted with chloroform (3x60 ml), extract dried with anhydrous Na_2SO_4 and solvent evaporated. Yield 180 mg (77 %), mp 138-140 °C (CH_2Cl_2/hexane). IR: 1628 (C=N), 3490 (N-H); ^{1}H NMR: 2.42 s, 3H, SCH_3; 3.82 s, 3H, OCH_3; 4.60 d, J=1 Hz, 2H, CH_2; 7.18 m, 4H and 7.72 m, 1H, 3-substituted indole, 8.19 br. s, 1H, NH; ^{13}C NMR: 14.26 (SCH_3), 45.95 (CH_2), 56.27 (OCH_3), 111.96, 116.50 (q), 120.21, 120.41, 122.80, 122.90, 127.92 (q), 137.48 (q), 158.33 (C=N). Anal. calcd. for $C_{12}H_{14}N_2OS$: C, 61.51; H, 6.02; N, 11.96; found: C, 61.39; H, 6.12; N, 11.88.

Brassitin ***3***. To a stirred solution of **28** (60 mg, 0.256 mmol) in tetrahydrofuran (6 ml) was added 1M HCl (two drops). After 3 min the solvent was evaporated, the residue dissolved in 20 ml of dichloromethane, filtered on charcoal and solvent evaporated. Yield 50 mg (87 %). The obtained amorphous **3** exhibited the spectral data (IR, ^{1}H and ^{13}C NMR as well as MS) fully identical with previously described data for brassitin.[10]

1-[N-(1-tert-Butoxycarbonylindol-3-ylmethyl)thiocarbamoyl]piperidine ***29***. To a solution of crude isothiocyanate **24c**, prepared from 1.48 mmol of amine **15c** in dichloromethane (20 ml) was added piperidine (0.219 g, 0.255 ml, 2.57 mmol) and the mixture was stirred at room temperature for 20 min. The solvent was evaporated and the residue chromatographed on 70 g of silica gel, eluent benzene/acetone (10:1). Yield 290 mg

(57 %), mp 96-98 °C ($CHCl_3$/hexane). IR: 1510 (NHCS), 1730(C=O), 3440 (N-H); ^{1}H NMR: 1.67 m, 15H, $(CH_3)_3$ and $(CH_2)_3$; 3.74 m, 4H, $N(CH_2)_2$; 5.00 d, J=4 Hz, 2H, CH_2; 5.51 br. t, J=4 Hz, NH; 7.30 m, 2H, 7.65 m, 2H and 8.17 m, 1H, 1,3-disubstituted indole. Anal. calcd. for $C_{20}H_{27}N_3O_2S$: C, 64.31; H, 7.29; 11.25; found : C, 64.20; H, 7.18; N, 11.37.

Acknowledgement: We thank the Slovak Grant Agency for Science (Grant No. 1/3229/96) for financial support of this work.

REFERENCES AND NOTES

1. Purkayastha, R. P. Progress in Phytoalexin Research During the Past 50 Years. In *Handbook of Phytoalexin Metabolism and Action*; Daniel, M.; Purkayastha, R. P. Eds.; Marcel Dekker: New York, 1995, pp. 1 39.
2. Lamb, C. J.; Ryals, J. A.; Ward, E. R.; Dixon, R. A. *Biotechnology* **1992**, 10, 1436-1445.
3. Hain, R.; Reif, H.-J.; Krause, E.; Langebartels, R.; Kindl, H.; Vornam, B.; Wiese, W.; Schmelzer, E.; Schreier, P. H.; Stöcker, R. H.; Stenzel, K. *Nature* **1993**, 361, 153-156.
4. Yoshikawa, M.; Tsuda, M.; Takeuchi, Y. *Naturwissenschaften* **1993**, 80, 417-420.
5. Afek, U.; Carmeli, S.; Aharoni, R. *Phytochemistry* **1995**, 39, 1347-1350.
6. Glazerbrook, J.; Zook, M.; Mert, F.; Kagan, I.; Rogers, E. E.; Crute, I. R.; Holub, E. B.; Hammerschmidt, R.; Ausubel, F. M. *Genetics*, **1997**, 146, 381-392.
7. Gross, D. *J. Plant. Dis. Prot.* **1993**, 100, 433-442.
8. Rouxel, T.; Kollman, A.; Belesdent, M.-H. Phytoalexins from Crucifers. In *Handbook of Phytoalexin Metabolism and Action*; Daniel, M.; Purkayastha, R. P. Eds.; Marcel Dekker: New York, 1995; pp. 229 261.
9. Gross, D.; Porzel, A.; Schmidt, J. *Z. Naturforsch.* **1994**, 49c, 281-285.
10. Monde ,K.; Takasugi, M.; Shirata, A. *Phytochemistry* **1995**, 39, 581-586.
11. Takasugi, M.; Katsui, N,; Shirata, A. *J. Chem. Soc., Chem. Commun.* **1986**, 1077-1078.
12. Takasugi, M.; Monde, K.; Katsui, N.; Shirata, A. *Bull. Chem. Soc. Jpn.* **1988**, 61, 285-289.
13. Monde, K.; Katsui, N.; Shirata, A.; Takasugi, M. *Chem. Lett.* **1990**, 209-210.
14. Takasugi, M.; Monde, K., Katsui, N., Shirata, A. *Chem. Lett.* **1987**, 1631-1632.
15. Monde, K.; Sasaki, K.; Shirata, A.; Takasugi, M. *Phytochemistry* **1991**, 30, 3921-3922.
16. Browne, L. M.; Conn, K. L.; Ayer, W. A.; Tewari, J. P. *Tetrahedron* **1991**, 47, 3909-3914.
17. Devys, M.; Barbier, M.; Loiselet, I.; Rouxel, T., Sarniquet, A.; Kollman, A.; Bosquet, J. F. *Tetrahedron Lett.* **1988**, 29, 6447-6448.
18. Monde, K.; Takasugi, M. *J. Chem. Soc. Chem. Commun.* **1991**, 1582-1583.
19. Monde, K.; Takasugi, M.; Ohnishi, T. *J. Am. Chem. Soc.* **1994**, 116, 6650-6657.
20. The first step in the biosynthesis of indole glucosinolates in oilseed rape and Chinese cabbage involve the formation of indole-3-carboxaldehyde oxime from L-tryptophan: Bennett, R.; Ludwig-Muller, J.; Kiddle, G.; Hilgenberg, W.; Wallsgrove, R. *Planta* **1995**, 196, 239-244.

21. Mehta, R. G.; Liu, J.; Constantinou, A.; Hawthorne, M.; Pezzuto, J. M.; Moon, R. C.; Moriarty, R. M. *Anticancer Res.* **1994**, 14, 1209-1214.
22. Mehta, R. G., Liu, J.; Constantinou, A.; Thomas, C. F.; Hawthorne, H.; You, M., Gerhäuser, C.; Pezzuto, J. M.; Moon, R. C.; Moriarty, R. M. *Carcinogenesis* **1995**, 16, 399-404.
23. Verhoeven, D. T. H.; Verhagen, H.; Goldbohm, R. A.; van den Brandt, P. A.; van Poppel, G. *Chem. Biol. Interact.* **1997**, 103, 79-129, and cited references.
24. Somei, M.; Kobayashi, K.; Shimizu, K.; Kawasaki, T. *Heterocycles* **1992**, 33, 77-80.
25. Putochin, N. *Ber. Deut. Chem. Ges.* **1926**, 59, 1987-2003.
26. Schallenberg, J.; Meyer, E. *Z. Naturforsch.* **1983**, 38b, 108-112.
27. Yamada, F.; Kobayashi, K.; Shimizu, A.; Aoki, N.; Somei, M. *Heterocycles*, **1993**, 36, 2783-2804.
28. Pedras, M. S. C.; Taylor, J. L. *J. Nat. Prod.* **1993**, 56, 731-738.
29. Shin, S.; Takahashi, N.; Yonezawa, Y. *Chem. Pharm. Bull.* **1990**, 38, 2020-2023.
30. Gribble, G. W.; Keavy, D.; Davis, D. A.; Saulnier, M. G.; Pelcman, B.; Barden, T. C.; Sibi, M. P.; Olson, E. R.; BelBruno, J. J. *J. Org. Chem.* **1992**, 57, 5878-5891.
31. Chauhan, P. M. S.; Chatterjee, R. K. *Indian J. Chem.* **1994**, 33B, 32-37.
32. Gower, B. G.; Leete, E. *J. Am. Chem. Soc.* **1963**, 85, 3683-3685.
33. For a recent review about synthetically useful reactions with nickel boride see: Khurana, J. M.; Gogia, A. *Org. Prep. Procedure Int.* **1997**, 29, 1-32, and cited references.
34. Velezheva, V. S.; Yarosh, A. V.; Kosik, T. A.; Suvorov, N. N. *Khim. Geterotsikl. Soedin.* **1978**, 1497-1503; *Chem. Abstr.* **1979**, 90, 87357.
35. Molina, P.; Alcántara, J.; López-Leonardo, C. *Tetrahedron Lett.* **1995**, 36, 953-956.
36. Molina, P.; Alcántara, J.; López-Leonardo, C. *Tetrahedron* **1996**, 52, 5833-5844.
37. Hanley, A. B.; Parsley, K. R. *Phytochemistry* **1990**, 29, 769-771.
38. Sharma, S. *Synthesis* **1978**, 803-820.
39. Staab, H. A.; Walter, G. *Liebigs Ann. Chem.* **1962**, 657, 104-107.
40. Jochims, J. C.; Seeliger, A. *Angew. Chem.* **1967**, 79, 151-152.
41. Stewart. J. J. P. MOPAC 6.0, QCPE Program 455.
42. Preliminary communication: Kutschy, P.; Achbergerová, I.; Dzurilla, M.; Takasugi, M. *Synlett* **1997**, 289-290.
43. Dhanak, D.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1986**, 2181-2186.
44. Wensbo, D.; Annby, U.; Gronovitz, S. *Tetrahedron* **1995**, 51, 10323-1042.
45. Doyle, K. J.; Moody, C. J. *Synthesis* **1994**, 1021-1022.
46. Kleinpeter, E.; Widera, R.; Mühlstädt, M. *J. Prakt. Chem.* **1978**, 320, 279-282.
47. Dzurilla, M.; Kutschy, P.; Koščík, D.; Toma, Š. *Collect. Czech. Chem. Commun.* **1990**, 55, 710-717.
48. Tandel, S. K.; Rajappa, S.; Pansare, S. V. *Tetrahedron*, **1993**, 49, 7479-7486.
49. Wackerle, L.; Ugi, I. *Synthesis* **1975**, 598-599.
50. Vangvearvong, S.; Nichols, D. E. *J. Org. Chem.* **1995**, 60, 3409-3413.
51. Suzuki, K.; Sekiya, M. *Chem. Lett.* **1979**, 1241-1244.