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Cancer chemopreventive activity of brassinin, a phytoalexin from cabbage

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Brassinin [3-(*S*-methyldithiocarbamoyl)aminomethyl indole], a phytoalexin first identified as a constituent of cabbage, was synthesized and evaluated for cancer chemopreventive activity. Dose-dependent inhibition of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced preneoplastic lesion formation was observed with mouse mammary glands in organ culture, as was dose-dependent inhibition of DMBA-induced mouse skin tumors that were promoted by treatment with 12-*O*-tetradecanoylphorbol-13-acetate. Cyclobrassinin is a biologically derived product of the oxidative cyclization of brassinin, and was as active as the parent compound in inhibiting the formation of preneoplastic mammary lesions in culture; however, 2-methylbrassinin was not significantly active in this process. Therefore, oxidative cyclization may be an effective metabolic activation step. As judged by these tumor inhibition studies in conjunction with potential to induce phase II enzymes in mice or cell culture, brassinin may be effective as a chemopreventive agent during both the initiation and promotion phases of carcinogenesis. This is the first report documenting the chemopreventive potential of structurally novel indole-based phytoalexins that are naturally occurring in cruciferous vegetables, and the synthetic route described herein has proven amenable for scale-up production. The bifunctional structural nature of brassinin, bearing both an indole nucleus and a dithiocarbamoylaminomethyl moiety, is notably similar to the individual structural elements of other known chemopreventive agents such as indole-3-carbinol or benzylisothiocyanate. The favorable biological activity demonstrated by the compound may originate from the presence of these two moieties.

Introduction

Numerous epidemiological studies have shown an association between reduced cancer risk and increased intake of green and yellow vegetables (1–3). This group of food plants largely falls within the Cruciferae and specifically within the genus *Brassica*. Examples included in this genus are cauliflower, cabbage, Brussels sprouts and broccoli. In experimental carcinogenesis studies, it has been shown that isothiocyanates or indole derivatives derived from tryptophan, such as indole-3-carbinol found in cruciferous vegetables, provide protection against chemical carcinogens (4–7).

Several tryptophan-derived, sulfur-containing phytoalexins have been isolated from vegetables of the Cruciferae (8–10). These agents demonstrate strong antifungal activity and are responsible for imparting resistance against such organisms (11). Chinese cabbage (*Brassica campestris* ssp. *pekinensis*) has been shown to contain at least eight isothiocyanate glucosinolates, including 3-indolylmethyl [NCS], identified as the aglycone (12). Exposure of Chinese cabbage to the bacterium *Pseudomonas chicorii* resulted in the production of three phytoalexins, namely, brassinin (**1**), 1-methoxybrassinin (**2**) and cyclobrassinin (**3**) (8,9).

The biosynthesis of brassinin involves enzymatic hydrolysis by myrosinase (thioglucosidase glucohydrolase) of the precursor glucosinolate, glucobrassicin (**4**), which undergoes a Lossen-type rearrangement to yield indolyl-3-methylisothiocyanate (**5**). Donation of a methyl group from methionine then yields brassinin (**1**), which, in turn, is converted to cyclobrassinin (**3**) (10). Alternatively, loss of $[NCS]^-$ from **5** yields **6**, which can subsequently add water to yield indole-3-carbinol (**7**), a compound that has been studied previously as an anti-carcinogen (3) (Scheme 1).

The isocyanato-group is a key functionality (13,14) contained in a number of anti-carcinogenic compounds such as 1-naphthyl isothiocyanate (13–16), benzyl isothiocyanate (17,18) and phenylalkyl isothiocyanate (19,20). Recently, $CH_3SO(CH_2)_4NCS$, given the trivial name sulforaphane, was isolated from broccoli and shown to induce NADPH:quinone oxidoreductase (quinone reductase) (EC 1.6.99.2) and glutathione-*S*-transferase (EC 2.5.1.1.18) activities in mouse tissue (19).

Based on the stuctural characteristics of brassinin and related structures, we were interested in exploring the chemopreventive potential of these compounds. Although brassinin (**1**) and cyclobrassinin (**3**) are naturally occurring, it would be very difficult to isolate sufficient quantities to investigate their possible chemopreventive role in experimental carcinogenesis models. In this report we describe the chemical synthesis of brassinin and cyclobrassinin. The chemopreventive efficacy of these compounds was assessed by monitoring inhibition of 7,12-dimethylbenz[*a*]anthracene (DMBA*)-induced mammary lesion formation in organ culture. In addition, the effect of brassinin in the two-stage mouse skin carcinogenesis model was determined.

Materials and methods

Brassinin was first synthesized from 3-aminomethylindole by reaction with carbon disulfide in the presence of triethylamine followed by treatment with iodomethane in pyridine (8). Syntheses of the key intermediate, 3-aminomethylindole, from indole-3-carboxaldehyde oxime, 3-cyanoindole or indole-3-carboxaldehyde (21,22), have been described as being poorly reproducible and giving low yields (21,23,24). The published synthesis of 3-aminomethylindole (21,22) worked rather poorly on scale-up. Therefore, we developed a high-yield, simple one-pot synthesis starting from commercially available indole-3-carboxaldehyde for brassinin (**1**) or 2-methylindole-3-

*Abbreviations: DMBA, 7,12-dimethylbenz[*a*]anthracene; QR, quinone reductase; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

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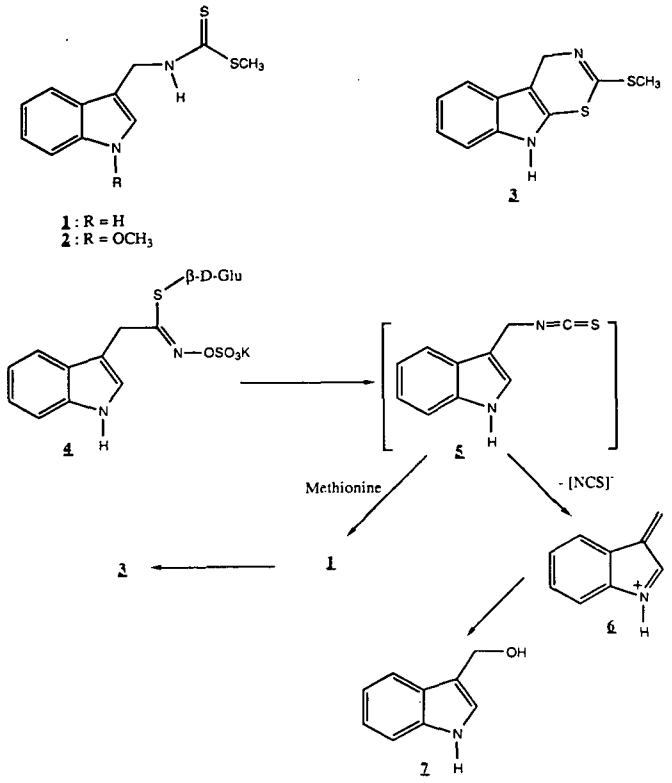
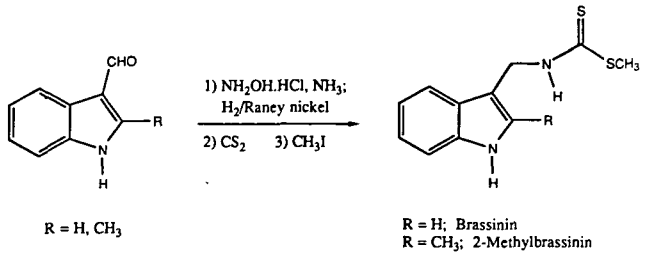
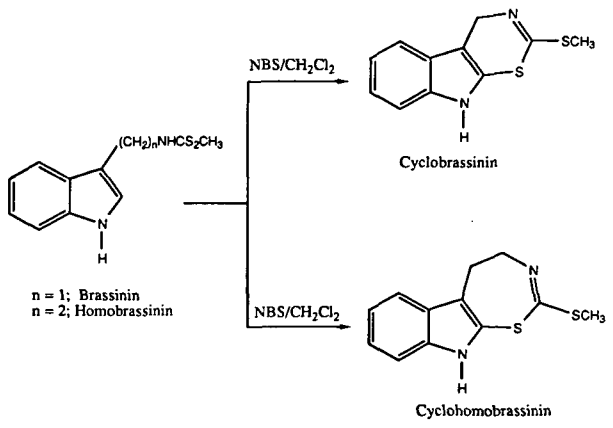


Scheme 1. Biosynthesis of brassin, cyclobrassin and indole-3-carbinol.



Scheme 2. Synthesis of brassinin.



Scheme 3. Synthesis of cyclobrassinin.

carboxaldehyde for 2-methylbrassinin (Scheme 2). A new procedure was also developed for the synthesis of cyclobrassinin (**3**) and cyclohomobrassinin (Scheme 3).

Synthesis of brassinin

To 120 ml of methanol saturated with ammonia at 0°C were added hydroxylamine hydrochloride 2.3 g (33.1 mmol, dissolved in 25 ml methanol) and indole-3-carboxaldehyde 4 g (27.5 mmol). The resulting mixture was shaken at room temperature for 2 h. Approximately 3 g of Raney nickel were added and the mixture was shaken at 45 p.s.i. hydrogen for 12 h. The catalyst was then removed by filtration and 1.66 ml (27.5 mmol) of carbon disulfide was added to the filtrate at 0°C. After 1 h, 1.72 ml (27.5 mmol) of iodomethane was added at 0°C, and the resulting mixture was stirred at room temperature for 2.5 h. The solvent was then evaporated *in vacuo* and the residue was extracted with 250 ml of ethyl acetate. The ethyl acetate solution was washed with 80 ml of 0.5 N hydrochloric acid twice, and then 100 ml water followed by 100 ml of saturated sodium chloride solution. After drying with sodium sulfate and filtration, the ethyl acetate was evaporated *in vacuo* to give a light-brown solid, which was recrystallized from 25 ml of benzene to give 4.7 g of cream-colored solid in 72% yield, m.p. 132–133°C [lit. (8) m.p. 132–133°C] and correct ^{1}H NMR and ^{13}C NMR spectra (8).

2-Methylbrassinin was synthesized using the above procedure from 1.56 g (10 mmol) of 2-methyl-3-indolecarboxaldehyde in a yield of 34%, m.p. 120–121°C. ^{1}H NMR (400 Hz, $CDCl_3$): d = 8.03 (s, 1H, br, 1-H), 7.52 (d, 1H, J = 7 Hz, 4-H), 7.32 (d, 1H, J = 7 Hz, 7-H), 7.19 (t, 1H, J = 7 and 7 Hz, 6-H), 7.14 (t, 1H, J = 7 and 7 Hz, 5-H), 6.85 (s, 1H, br, N-H), 4.97 (d, 2H, J = 4 Hz, -CH_2-), 2.61 (s, 3H, S-CH_3), 2.40 (s, 3H, 2-CH_3). The spectrum showed minor signals (about one-quarter the intensity of the major ones) due to a rotamer at d = 4.67 (d, 2H, J = 4 Hz, -CH_2-) and 2.74 (s, 3H, -SCH_3). MS: *m/z* (%) = 250 (27, M^+). Anal. calc. for $C_{12}H_{14}N_2S_2$: C, 57.57; H, 5.64; N, 11.19. Found: C, 57.43; H, 5.59; N, 10.99.

Synthesis of cyclobrassinin

N-Bromosuccinimide 0.18 g (1 mmol) dissolved in 12 ml of dichloromethane was added dropwise to a solution of brassinin 0.24 g (1 mmol) in 10 ml of dichloromethane at 30°C and stirred for 5 min.

After cooling to room temperature, 0.28 ml (2 mmol) of triethylamine was added. The solvent was evaporated *in vacuo*, and the residue was then applied to a silica-gel column and eluted with 2:1 petroleum either:dichloromethane. The first fraction was evaporated and recrystallized from dichloromethane to give a white solid (0.08 g) in a yield of 34%, m.p. 136–137°C with correct ^{1}H NMR and ^{13}C NMR spectra (8).

Mouse mammary gland organ culture

Young, virgin BALB/c female mice 3–4 weeks of age were obtained from Charles River (Wilmington, MA). Mice were treated daily with 1 µg of estradiol plus 1 mg of progesterone for 9 days to prepare mammary glands for culture (25). Mammary gland organ culture procedures have been described previously (25–27). Briefly, mice were killed by cervical dislocation and thoracic pairs of mammary glands were dissected and explanted on silk rafts. The glands were incubated for 10 days in chemically defined, serum-free Waymouth MB752 medium supplemented with insulin, prolactin, aldosterone and hydrocortisone. During this 10 day period, brassinin was included in the growth-promoting medium in concentrations ranging from 10^{-9} to 10^{-5} M. Fifteen glands were used in each group. The carcinogen, DMBA (2 µg/ml) was added to the medium between days 3 and 4 to generate mammary lesions. After 10 days of growth phase, the glands were incubated for an additional 14 days with only insulin added to the medium. During the entire culture period the glands were maintained at 37°C under a 95% O_2/5% CO_2 environment. At the end of the exposure, the glands were fixed in formalin, stained in alum carmine and scored for mammary lesions.

Quinone reductase (QR) activity

Mammary glands were incubated with insulin, prolactin, aldosterone and hydrocortisone for 3 days as described above. Brassinin or cyclobrassinin was included in the medium at a concentration of 50 µg/ml. Individual mammary glands were homogenized in 0.1 M phosphate buffer, pH 6.5. The supernatant was collected by centrifuging the homogenate at 20 000 *g* for 5 min. QR activity in the mammary gland supernatant was measured as described in the literature (19).

Skin carcinogenesis

CD-1 male mice 4–5 weeks of age were obtained from Charles River breeding laboratories. After 1 week of quarantine all mice were shaved. Animals showing regrowth of hair, nicks and cuts were removed from the study. Animals were randomized into seven groups as summarized in Table I. Animals were treated by applying 50 µg DMBA in 0.2 ml acetone as a single application. The mice were treated with 2.5 ng of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 0.2 ml of acetone twice weekly for 12 weeks.

Brassinin was dissolved in acetone (0.2 ml) and applied to the backs of mice at concentrations of either 1 or 2 µg (0.5 or 1%) twice weekly for 12 weeks. Brassinin was applied 1 h prior to TPA treatment. Animals were weighed daily and observed for tumor development; records were maintained for the location and number of tumors in mice. This protocol is essentially the same as described previously (28–30).

Table I. Protocol to determine potential inhibition of CD-1 mouse skin tumorigenesis by brassinin

Group	No. of mice	DMBA (μg)	TPA (μg)	Brassinin (0.2 ml)
1	20	none	none	none
2	20	vehicle	none	1%
3	20	50	2.5	none
4	20	50	2.5	vehicle
5	20	50	2.5	1%
6	20	50	2.5	0.5%
7[a]	20	50	2.5	1%

[a]Group 7: animals received five additional doses of 1% brassinin prior to DMBA dosing.

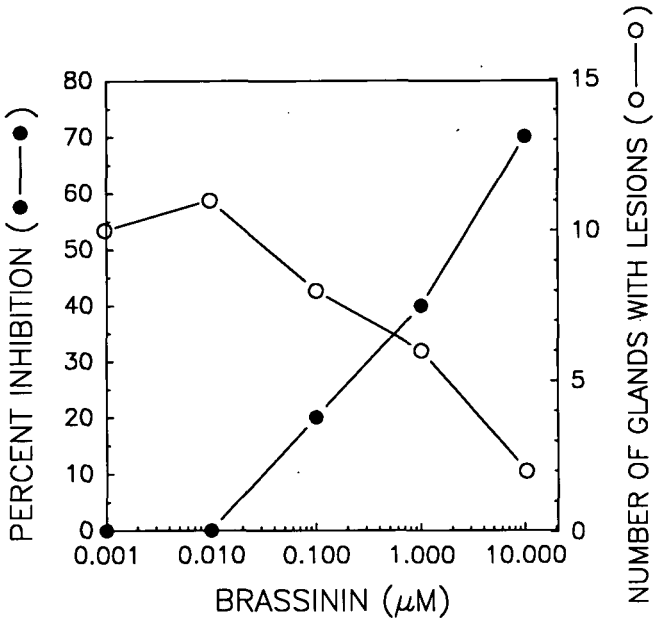


Fig. 1. Inhibition of DMBA-induced mammary lesions by brassinin. Thoracic pairs of mammary glands were incubated with growth-promoting hormones in the presence of increasing concentrations of brassinin for 10 days. The glands were incubated for an additional 14 days with insulin-containing medium. DMBA was included in the medium for 24 h on the third day of culture. Fifteen glands were used for each concentration point. The glands were stained and scored for mammary lesions. Per cent inhibition was calculated. Statistical significance was determined by chi-square analysis. Inhibition of 60% or greater is statistically significant ($P < 0.05$) as compared to controls.

Results

Inhibition of DMBA-induced mammary lesions

The chemopreventive effect of brassinin and some analogs was first evaluated in the mouse mammary gland organ culture model. Mammary glands respond to DMBA to form preneoplastic, hyperplastic alveolar nodule-like lesions under appropriate hormonal conditions. These alveolar structures are termed mammary lesions (26), since transplantation of epithelial cells derived from these lesions forms adenocarcinomas in syngeneic mice (31). Inhibition of mammary lesion development by chemopreventive agents has been established to predict the chemopreventive potential of active agents in *in vivo* experimental carcinogenesis systems for mammary tumors (26,32).

Mammary glands from young female mice were incubated with brassinin in concentrations ranging from 10^{-9} to 10^{-5} M during the first 10 days of the proliferation phase in the presence of growth-promoting hormones. At the termination of the experiment, the glands were stained with alum carmine and examined for the presence of lesions. Brassinin had no inhibitory effect at the two lowest concentrations of 10^{-9} and 10^{-8} M. However, at higher concentrations, brassinin inhibited mammary lesion formation in a dose-dependent manner (Figure 1). At 10^{-5} M, there was a 70% reduction in the number of mammary glands with lesions as compared to control glands that were incubated with vehicle. These results suggested that brassinin was an effective chemopreventive agent in this experimental model. No toxicity was observed in the concentration range employed, i.e. dilatation and disintegration of mammary ducts were not observed.

Table II. Effect of brassinin and its analogs on the development of DMBA-induced mammary lesions

Chemopreventive agent (1×10^{-5} M)	No. glands with lesions	% inhibition
None	10/15	–
Brassinin	2/15[a]	80.0
Cyclobrassinin	1/15[a]	90.9
2-Methylbrassinin	11/15	0

[a]Significantly different from control (chi-square analysis, $P < 0.05$).

In addition to brassinin, two analogs were evaluated for activity against the formation of mammary lesions, cyclobrassinin and 2-methylbrassinin. It was anticipated that comparison of effects mediated by these compounds could provide insight regarding structure–activity relationships on the mode of action for brassinin. Cyclobrassinin was highly effective in inhibiting the development of mammary lesions. At 10^{-5} M, this compound inhibited the incidence of mammary lesions by 90%, which was comparable with that of brassinin. The apparent increased inhibition by cyclobrassinin as compared to brassinin is not statistically significant. However, 2′-methylbrassinin was not significantly active at the same concentration (Table II).

The effect of brassinin and cyclobrassinin on the induction of QR activity was measured. Mammary glands were incubated with brassinin and its analog for 3 days in the presence of growth-promoting hormones, As shown in Table III, brassinin (50 μg/ml, 2.2×10^{-4} M μM) induced QR activity by 4-fold. The mean specific activity in brassinin-treated glands was 166 units as compared to 40 units in the control glands. Cyclobrassinin at the same concentration induced QR activity by 29-fold. This is a remarkable enhancement as compared to controls, as well as induction of the enzyme by brassinin.

Inhibition of skin carcinogenesis

The effects of brassinin were evaluated in a two-stage skin carcinogenesis assay with CD-1 mice. During the 90 day study, 95% incidence was observed in the mice treated with both DMBA and TPA. Brassinin, at a concentration of 1%, reduced the tumor incidence to 50%, whereas animals treated with a 0.5% brassinin solution had a tumor incidence of 65% (Figure 2). In group 7, in which brassinin treatment was initiated prior to DMBA treatment and continued during the promotion phase of carcinogenesis, no additional protection was provided. The tumor incidence in this group of mice was 55%. Results in Figure 3 show effects of brassinin on tumor multiplicity. The number of skin tumors in mice treated with only carcinogen and promoter increased modestly during the 90 day period. In mice treated with a 1% brassinin solution,

Table III. Effect of brassinin and cyclobrassinin on the induction of QR activity in mammary gland organ culture

Chemopreventive agent (2.2×10^{-4} M)	QR activity (OD_{595}/mg protein)
None	39.88±9.80
Brassinin	165.52±42.88[a]
Cycobrassinin	1136.64±145.58[b]

Mammary glands were incubated with insulin, prolactin, aldosterone and hydrocortisone alone or in the presence of the chemopreventive agents for 3 days. Results represent a mean of the enzyme activity from five separate glands. The assays were carried out in triplicates.
[a]Statistically different from both the control and cyclobrassinin treatment groups (Student's *t*-test; $P < 0.001$).
[b]Statistically significant from the control (Student's *t*-test; $P < 0.001$).

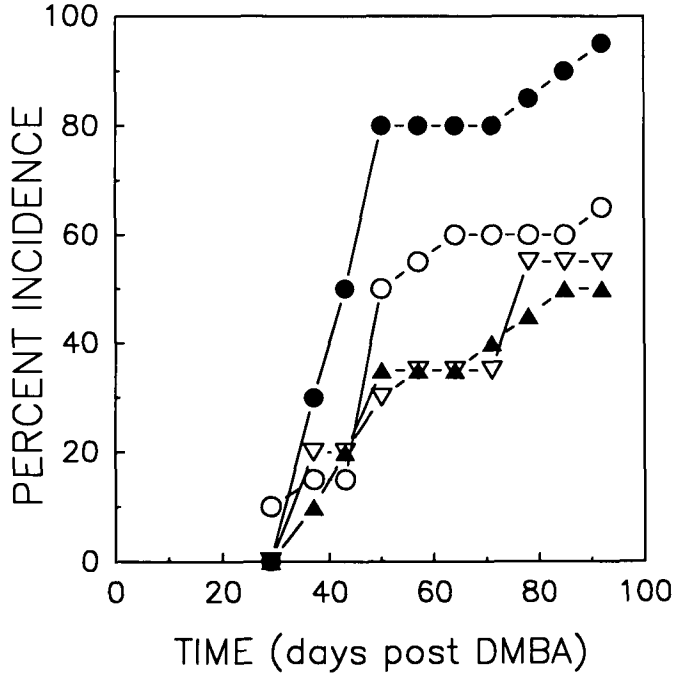


Fig. 2. Effects of brassinin on the incidence of skin tumorigenesis. Skin tumors were induced in a two-stage carcinogenesis model as described in the text. Animals were treated either with a single dose of 50 μg/ml DMBA and 2.5 μg TPA twice weekly for 12 weeks. Brassinin was topically applied on the skin either at 1% or 0.5% concentrations 1 h prior to TPA application. ●, control; ○, 0.5% brassinin; ▲, 1% brassinin; ▽, 1% brassinin (beginning 5 days prior to DMBA treatment until the end of the experiment).

the number of skin tumors was 5.4 per animal as compared to 17.5 in control mice. Animals treated with a 0.5% solution of brassinin exhibited 7.8 tumors per mouse. Again, treatment of animals during the initiation and promotion phases did not show any additional protection as compared to treatment only during the promotion phase (Table IV). In the present study, histopathological evaluations were not made on the skin tumors, and therefore, the effect of brassinin on the incidence of skin carcinoma cannot be determined. However, it has been previously reported that the skin tumors formed using this protocol are largely papilloma. Verma *et al.* showed that under these conditions there was no carcinoma found during the first 25 weeks of treatment with TPA (33). Since the present study was terminated at 90 days (13 weeks), it can be expected that the majority of the tumors were papilloma. No brassinin toxicity was observed. There was a 31% increase in body

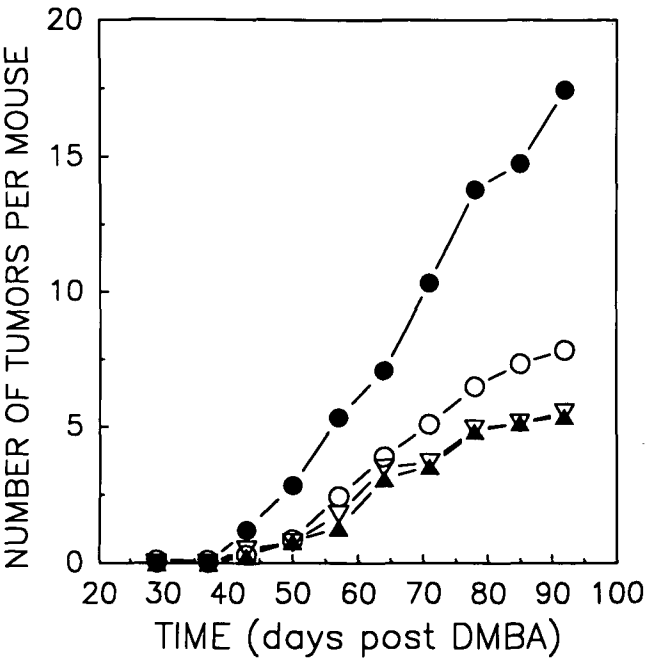


Fig. 3. Effects of brassinin on the multiplicity of skin carcinogenesis. Skin tumors were induced in a two-stage carcinogenesis model as described in the text. Animals were treated either with a single dose of 50 μg DMBA and 2.5 μg TPA twice weekly for 12 weeks. Brassinin was topically applied on the skin either at 1% or 0.5% concentrations 1 h prior to TPA application. ●, control; ○, 0.5% brassinin; ▲, 1% brassinin; ▽, 1% brassinin (beginning 5 days prior to DMBA treatment until the end of the experiment).

weight of controls during the 90 day experiment period as compared to a 33.0% increase in body weight in the group treated with brassinin at a concentration of 1%.

Discussion

In recent years, considerable attention has been focused on increased dietary intake of vegetables and fruits since, epidemiologically as well as experimentally, such a diet has been positively correlated with reduced risk of developing cancer (1–3). Among vegetables, broccoli, cauliflower, cabbage and Brussels sprouts contain isothiocyanates and indole derivatives, which have shown chemopreventive activity against a variety of experimental tumor models (5–7). Indole-3-carbinol, 3,3′-diindolylmethane and indole-3-acetonitrile are three notable substances that have been identified in cruciferous vegetables (3). Indole-3-carbinol has been most intensely studied, but the chemopreventive potential of this compound remains ambiguous (5,34,35). In trout, for example, indole-3-carbinol was effective against the initiation of carcinogen-induced hepatocarcinogenesis, but enhancement was observed in the promotion phase (36,37).

Brassinin was first isolated from Chinese cabbage (9) as a phytoalexin, and its antifungal properties have been documented (11,12). However, very little information is available on the activity of brassinin or its analogs in other biological systems. Cyclobrassinin, brassilexin, 5-methoxybrassilexin and homocyclobrassinin were evaluated as growth inhibitors with cultured KB cell s. It was found that brassilexin was the most effective, inhibiting KB cell growth at a concentration of 8 μg/ml, while cyclobrassinin was less effective. The chemopreventive activity of brassinin or related analogs has not been reported previously, but was of interest due to the structural

Table IV. Effect of brassinin on the incidence and multiplicity of DMBA-induced, TPA-promoted skin tumors in CD-1 mice

Group	No. of animals	Treatment	% incidence	No. of tumors per mouse	Terminal body wt (g)
1	20	basal diet	0.0	0.0	26.5
2	20	DMBA vehicle + 1% brassinin	0.0	0.0	27.0
3	20	DMBA + TPA	95.0	17.5	27.1
4	20	DMBA + TPA + brassinin vehicle	90.0	16.2	26.3
5	20	DMBA + TPA + 1% brassinin	50.0[a]	5.4[a]	27.4
6	20	DMBA + TPA + 0.5% brassinin	65.0	7.8[b]	26.2
7	20	DMBA + TPA + 1% brassinin (−2 to end)	55.0[a]	5.6[b]	26.6

Statistical comparison of incidence was made using chi-square analysis; the multiplicity was compared using Student's *t*-test.
[a]Significantly different from control groups 3 and 4 ($P < 0.03$).
[b]Significantly different from control groups 3 and 4 ($P < 0.01$).

characteristics of this substance. Since brassinin occurs in low abundance as a phytoalexin, the first task at hand was to obtain a sufficient quantity of material for biological evaluation. The synthetic schemes reported in this paper make it possible to obtain multi-gram quantities of these agents. With the availability of these materials, one of the first studies undertaken was inhibition of preneoplastic lesion generation in mammary organ culture.

Mouse mammary gland organ culture has been employed as a tool to evaluate newly discovered agents for their effectiveness against the development of DMBA-induced mammary lesions. Examination of >100 potential chemopreventive agents has resulted in strong correlation between effectiveness in the organ culture model and *in vivo* mammary carcinogenesis (26). As currently reported, brassinin and cyclobrassinin inhibited mammary lesion development by >60% ($P < 0.05$). Therefore, since these results are strongly suggestive of chemopreventive activity, additional studies were performed with animal models. Accordingly, using the standard two-stage mouse skin model, compelling evidence for chemopreventive activity was obtained, and experiments that are currently in progress have indicated that brassinin also inhibits DMBA-induced mammary carcinogenesis in rats.

Thus, our initial speculation regarding the chemopreventive potential of brassinin was verified, and it became of interest to investigate its mode of action. Wattenberg has classified cancer chemopreventive agents into three major categories: (i) agents that prevent carcinogen formation; (ii) blocking agents that may either inhibit enzymes necessary for carcinogen metabolism, cause carcinogen detoxification or scavenge the reactive carcinogen prior to its action at the target organ; and (iii) suppressing agents that are effective during tumor promotion (2,39). In this context, it appears that brassinin may have anti-carcinogenic activity during both the initiation and promotion phases of carcinogenesis. As shown in Table IV, brassinin inhibited DMBA-induced and TPA-promoted skin carcinogenesis when present only during the TPA treatment phase. Additional protection was not afforded when brassinin was present during both the initiation and promotion phases of the experiment. These data support inhibition during the promotion phase but do not preclude inhibition during the initiation phase.

A variety of mechanisms may contribute to anti-initiating activity, but of key importance may be the induction of phase II enzymes that metabolically inactivate chemical carcinogens. For example, Talalay and co-workers have recently identified sulforaphane as a constitutuent of broccoli that is capable of inducing QR and glutathione-*S*-transferase (19,40). As shown in Table III, both brassinin and cyclobrassinin induced QR activity by 4- and 29-fold respectively. However, it should be pointed out that the QR activity was induced by a 20-fold higher concentration of cyclobrassinin than required to inhibit formation of mammary lesions in culture. Similarly, when administered to mice, glutathione-*S*-transferase activity was also induced in the rat liver (L.K.T.Lam, unpublished observations). Thus although speculative, it is possible that the anti-initiating activity of brassinin and cyclobrassinin observed in the present study may be mediated by induction of phase II enzymes such as QR. Indole-derived compounds such as indole-3-carbinol often induce phase I specific enzymes in addition to phase II enzymes (36,37). This has led to some concerns about the use of indole-3-carbinol as a cancer chemopreventive agent. However, we have not evaluated effects of brassinin and its analogs on carcinogen metabolism specific enzymes.

The potential mechanisms by which these compounds may inhibit carcinogenesis at the stage of promotion are less well characterized. We have determined that these compounds do not inhibit the catalytic activity of protein kinase C, do not inhibit ornithine decarboxylase activity induced by treatment of cultured mouse 308 cells with phorbol esters, and do not induce cell differentiation as judged by cultured HL-60 cells.

An additional consideration worthy of comment deals with the metabolism of brassinin. Relative to brassinin, greater induction of QR activity was observed with cyclobrassinin. Consistent with these results is the hypothesis that brassinin may be cyclized to cyclobrassinin in order to mediate activity. However, we have thus far been unable to confirm this possibility with mammary organ culture.

To conclude, this is the first report which provides evidence for the chemopreventive activity of structurally novel, indole-based phytoalexins that are naturally occurring in cruciferous vegetables. Large-scale synthetic production of additional material is currently under way which will permit assessment of efficacy and additional mechanistic evaluations. The unique structural features of these compounds, bearing both the indole nucleus and the isothiocyanate-based side chain, provide good promise for their use as chemopreventive agents.

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