Biochemical Pharmacology, Vol. 37, No. 23, pp. 4479–4486, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

ANTI-ASTHMATIC EFFECTS OF ONIONS

ALK(EN)YLSULFINOTHIOIC ACID ALK(EN)YL-ESTERS INHIBIT HISTAMINE RELEASE, LEUKOTRIENE AND THROMBOXANE BIOSYNTHESIS *IN VITRO* AND COUNTERACT PAF AND ALLERGEN-INDUCED BRONCHIAL OBSTRUCTION *IN VIVO**

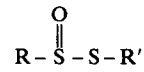
W. DORSCH,†‡ H. WAGNER,§ TH. BAYER,§ B. FESSLER,§ G. HEIN,‡ J. RING,‖ P. SCHEFTNER,‡ W. SIEBER,‡ TH. STRASSER¶ and E. WEIß‡

‡ Pediatric Department of the Poliklinik, § Institute for Pharmaceutical Biology, ¶ Department of Internal Medicine, ‖ Department of Dermatology, Ludwig-Maximilians-University, Munich, Federal Republic of Germany

(*Received* 10 *September* 1987; *accepted* 18 *June* 1988)

Abstract—Five alk(en)ylsulfinothioic acid alk(en)yl-esters isolated from onions and four synthetic thiosulfinates inhibited 5-lipoxygenase of porcine leucocytes, histamine release and leukotriene B_4 and C_4 biosynthesis of human polymorphonuclear leucocytes, thromboxane B_2 biosynthesis by human platelets and allergen- and PAF-induced bronchial obstruction of guinea-pigs.

The anti-asthmatic and anti-inflammatory effects of onions depend in part on the thiosulfinate moiety:



$$R-\overset{\overset{O}{\|}}{S}-S-R'$$

Most of anti-asthmatic agents used today, such as β-sympathomimetics, the xanthine theophyllin, anticholinergics and the khellin related disodium cromoglycate have been derived from natural products. In addition, a large number of plant extracts are used in traditional medicine for the treatment of inflammatory disorders. Thus the use of *Allium cepa* (onion) can be dated back to old Egyptian handscripts for its antimicrobial and anti-inflammatory properties [1]. Fresh onion juice is recommended by dermatologists for the treatment of inflammatory reactions to bee or wasp stings [2]. Inhibitory effects of onion oils on arachidonic acid metabolism and platelet aggregation are well known [3–5]. In 1983 we reported the anti-asthmatic and anti-inflammatory properties of onion extracts [6–8]. Recently, we found that lipophilic onion extracts counteract PAF-induced bronchial obstruction in guinea-pigs and inhibit thromboxane biosynthesis by human lung fibroblasts [9]. The present paper describes pharmacological properties of alk(en)yl sulfinothioc acid alk(en)yl esters (syn.: thiosulfinates) isolated from onion extracts and identified as its main or sole anti-asthmatic principle. This discovery was substantiated by pharmacological studies on synthetic sulfinothioic acid esters.

* Presented in part at the 17th Symposium of the Collegium Internationale Allergologicum and the 44th meeting of the American Academy of Allergy and Immunology, 1988.

† Present address: Prof. Dr. W. Dorsch, Kinderklinik, Langenbeckstr. 1, D-6500 Mainz, F.R.G.

MATERIALS AND METHODS

Isolation and structure elucidation of alk(en)ylsulfinothioic acid alk(en)yl-esters

Preparation of lipophilic onion extracts. Allium cepa bulbs were peeled, chopped, and after standing for about 30 min at room temperature squeezed to give onion juice. OJC (onion juice chloroform extracted) was prepared by shaking the juice with chloroform and evaporating the solvent at reduced pressure. Lyophilized onion extract (LOE), lipophilic extract of LOE (LOE/A) and ether extracts of lyophilized onions (LOI) were prepared as described previously [9].

Fractionation of OJC. Triterpenes were removed by flash chromatography on reversed-phase material with methanol as solvent. Crude fractionation using a rotating disk chromatography system (silica gel coated disk, chloroform as solvent) provided the fractions OJC I/II/III and IV. OJC II was separated by column chromatography (silica gel, solvent system—toluene/ethyl acetate 10:2) into 5 subfractions: OJC II 0/1/2/3 and 4. The most active fractions OJC II 2 and OJC II 3 each showed three major peaks in the HPLC. The peaks were numbered according to their retention time. Spectroscopical investigations showed that peak 2/3 contained two compounds (2/3-1 and 2/3-2).

Isolation of active compounds. The pure compounds OJC II 2/1, 2/2, 2/3-1, 2/3-2, 3/3 and the mixture of OJC II 3/(1 + 2) were obtained using middle pressure liquid chromatography (MPLC);

stationary phase—silica gel, mobile phase—dichloromethane/acetone 100:1 (v/v).

Identification of the isolated compounds. The identification and structure elucidation was performed by means of elementary analysis, UV-, mass-, proton (^1H) and carbon (^{13}C) NMR-spectroscopy. More detailed information concerning the isolation as well as the structure elucidation will be published elsewhere.

Synthesis of thiosulfinates

Homologue thiosulfinates were synthesized as described [10]: Symmetric disulfides are oxidized by perbenzoic acid in an organic solvent to provide the corresponding thiosulfinates. The synthetic procedure was monitored by TLC and the products were identified by spectroscopic methods. The thiosulfinates were stored immediately after isolation or synthesis under nitrogen and at −20° to avoid decomposition.

Pharmacological studies in vitro

Effects on 5-lipoxygenase of porcine leucocytes. 5-Lipoxygenase activity was tested as described by Kuhl *et al.* [11]: a suspension of porcine leucocytes in phosphate buffer (10^7 cells/ml) was incubated with calcium chloride, ETYA, genuine and synthetic compounds, calcium ionophore A 23187 and 1-^{14}C-arachidonic acid for 5 min at 37°. The reaction was terminated by the addition of formic acid. Arachidonic acid and its metabolites were extracted by ethylacetate. Separation and quantification was performed by reversed-phase HPLC using an acetonitrile/water gradient system [11]. The labelled compounds were detected and quantified by measurement of radioactivity.

Effects on leukotriene biosynthesis of human peripheral leucocytes. Human polymorphonuclear leucocytes were prepared by centrifugation over a Percoll density gradient as described [12]. The leucocyte-to-platelet ratio was above 90:1 and viability of the cells was about 97% after several washing procedures [13]. All synthetic compounds were preincubated in two-fold dilutions with purified cells for 10 min in a volume of 1 ml RPMI 1640 (Sigma, F.R.G.) with added Ca^{2+} (final concentration 1.8 mM) and stimulated with the calcium ionophore A 23187 (10 μM) for 5 min. After extraction of the supernatant with SepPack cartridges analysis by HPLC was carried out as described [13].

Effects on thromboxane B_2 biosynthesis of human platelet rich plasma. Citrated plasma was harvested from atopic volunteers. Platelet rich plasma (PRP) was adjusted at 90,000 platelets per ml in K_2HPO_4-buffer. 1 ml PRP was incubated for 3 min in duplicate with four different concentrations (1 mg/ml–1 μg/ml) of LOI, OJC, its subfractions and synthetic compounds dissolved in 1% DMSO (Sigma, St. Louis, MO). Stimulation was done with 10 or 1 I.U. thrombin (Behringwerke, Marburg, F.R.G.). After 15 min the reaction was stopped by adding ice-cold methanol. Thromboxane B_2 in the supernatant was determined by RIA in triplicate corrected by recovery rates (80–100%) according to the method of Siess *et al.* [14].

Effects on histamine release from human peripheral leucocytes in vitro. Venous blood was taken from 11 atopic donors. Peripheral leucocytes were isolated by dextran sedimentation and preincubated with four different concentrations (0.1–100 μM) of the synthetic compounds or 1% ethanol for control. Ten minutes later they were challenged with 2 I.U. rabbit anti-human IgE antibodies (Behringwerke, Marburg, F.R.G., reverse anaphylaxis) or saline (control experiments for measuring histamine release by thiosulfinates) for 30 min. Histamine in the supernatant was measured spectrofluorometrically [15], the results being expressed as percentage of maximal (= 100%) release after cell disruption by perchloric acid, corrected for the spontaneous release without stimulation (<5%).

Pharmacological studies on guinea pigs in vivo

Animals. Male Pirbright white guinea-pigs (200–250 g) were purchased from Ivanovas, Kisslegg, F.R.G., and sensitized to ovalbumin (OA) as described [16]. Experiments were done 3–4 weeks later (bodyweight about 300–400 g).

Experimental design. All experiments were performed according to a randomized crossover protocol [7]: groups of 8–20 animals were divided into two subgroups and treated either with the compound tested or vehicle only (control). Forty-five minutes later the animals inhaled allergen solutions (twice 30 or 60 sec at time 0 and 10 min), platelet-activating factor (once 60 sec), histamine (30 sec) or acetylcholine (30 sec). Two or three days later the tests were repeated, animals prior treated with control receiving the tested compound and vice versa.

Administration of extracts and compounds. Onion extracts and onion-derived compounds were dissolved in 0.5 ml olive oil (Carl Roth KG, Karlsruhe, F.R.G.) and given orally by tuberculin syringes. Ovalbumin (OA, grade III, Sigma, St. Louis, MO), histamine hydrochloride (H, Sigma), and acetylcholine (Ach, Sigma) were dissolved in saline (1:99, 1:999, 1:99, w/v); platelet activating factor (PAF, C-16-racemate, Fa. Nattermann, Cologne, F.R.G.) was first dissolved in 25 μl ethanol and then in 10 ml saline containing 0.25% (w/v) bovine serum albumin (Sigma) yielding a final concentration of 1 μg PAF/ml. PAF, OA, H and Ach were nebulized ultrasonically (Heyer, USE 77, Bad Ems, F.R.G.).

Lung function method. The animals' lung function was tested prior to and sequentially after inhalation challenges by two chambered body plethysmography using the amount of "compressed air" (ml) as parameter. This non-invasive method has been shown to be highly sensitive in monitoring bronchial obstruction [16].

Statistics

All figures show mean ($\bar{x}$) and standard deviation (SD). If not mentioned otherwise, the statistical significance of differences was estimated using Student's *t*-test for unpaired data. In addition, the inhibitory activity of several compounds was expressed by comparing maximal values of compressed air after placebo treatment (100%) with maximal compressed air values after verum treatment (x%). The degree of "per cent inhibition" ($100 - x$%) was calculated for each animal sep-

arately and will be given as mean ± standard deviation ($\bar{x}$ ± SD). Statistical significance was estimated using Student's *t*-test for paired data.

RESULTS

Biological effects of crude lipophilic onion fractions

LOI (ether extract of lyophilized onions) inhibited allergen induced bronchial obstruction of seven guinea-pigs down to a dose of 20 mg/kg ($P < 0.02$, per cent inhibition = 64.3 ± 29%), more effectively than a chloroform extract of lyophilized onions (LOE/A). A chloroform extract of fresh onion juice (OJC) dampened asthmatic reactions of guinea-pigs to allergen inhalation challenges much more than the whole onion (lyophilized onion extract = LOE, Fig. 1). OJC prevented PAF-induced bronchial obstruction (Fig. 2). It showed more than 30 major peaks (detection at 200 nm) in the HPLC chromatogram (Fig. 3).

Biological effects of lipophilic subfractions

OJC was divided into four major fractions and tested for biological activity. Fractions OJC I, OJC III, OJC IV as well as triterpenes removed from OJC by flash chromatography did not alter bronchial reactions of 8–15 guinea-pigs, neither to allergen nor to PAF-inhalation. Fraction OJC II significantly

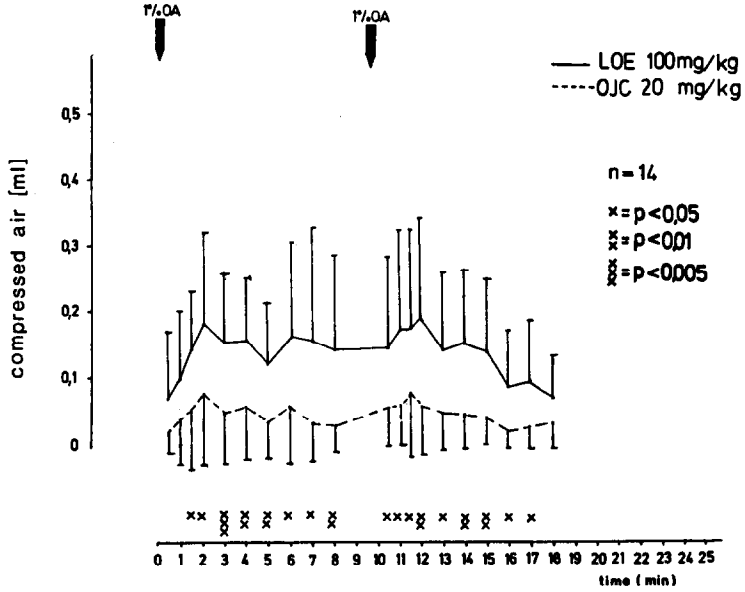


Fig. 1. 20 mg/kg OJC (= onion juice chloroform extracted) inhibits more effectively the allergen-induced bronchial obstruction of guinea-pigs than 100 mg/kg LOE (= lyophilized onion extract).

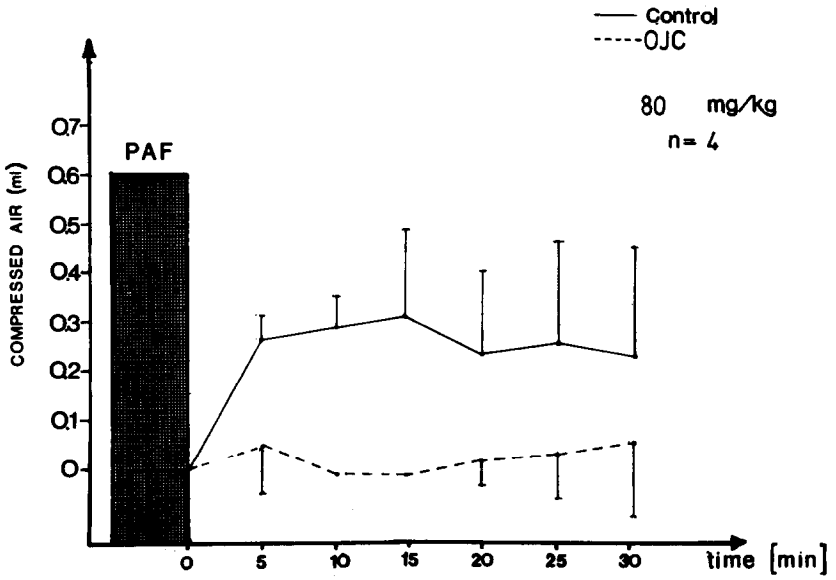


Fig. 2. Inhibition of PAF-induced bronchial obstruction in four guinea-pigs by oral treatment with 80 mg/kg OJC (= onion juice chloroform extracted).

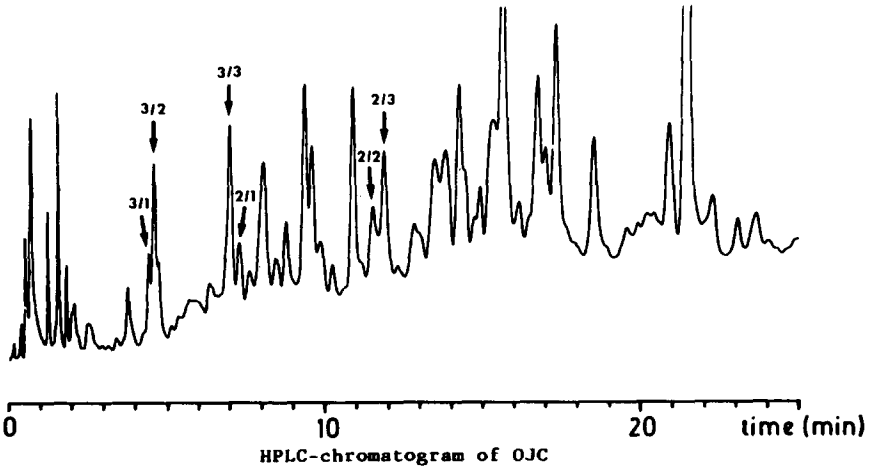


Fig. 3. HPLC chromatogram of onion juice chloroform extracted (OJC); stationary phase: LiChrospher 100 CH-18; column: Hibar, Merck, mobile phase: acetonitrile/water gradient system from 20% to 60% acetonitrile in 30 min; flow: 1 ml/min; detection at 200 nm.

diminished the PAF-induced bronchial obstruction of 9 guinea-pigs even at oral doses of 15 mg/kg (per cent inhibition: 55.1 ± 32.5%; $P < 0.005$; *t*-test for paired data). OJC II was further divided into five subfractions (0–4). Highest biological activity was found in fractions OJC II 2 and OJC II 3: 20 mg/kg fraction OJC II 3 reduced the reaction of nine guinea-pigs to allergen challenges by 47.1 ± 37.7% (1. challenge, $P < 0.01$) and 47.6 ± 37.2% (2. challenge, $P < 0.05$). The same dose of OJC II 2 demonstrated per cent inhibition rates of 76.8 ± 16.4% or 73.9 ± 17.1% ($P < 0.005$). Thromboxane biosynthesis of human platelets was diminished dose-dependently (Table 1).

Identification of alk(en)ylsulfinothioic acid-alk(en)ylesters and dithietane derivatives in lipophilic onion subfractions

Each of the active subfractions showed three major peaks in HPLC (Fig. 4). To them the following compounds could be assigned:

—OJC II 2/1: *trans* - 2,3 - dimethyl - 5,6 - dithiacyclo 2.1.1 heptane 5-oxide (dithietane derivative);

—OJC II 2/2: propylsulfinothioic acid-S-1-*cis*-propenyl-ester;

—OJC II 2/3: propylsulfinothioic acid-S-1-*trans*-propenyl-ester (2/3-1);
and:
propylsulfinothioic acid-S-propyl-ester (2/3-2);

—OJC II 3/1: methylsulfinothioic acid-S-1-*trans*-propenyl-ester;

—OJC II 3/2: methylsulfinothioic acid-S-1-*cis*-propenylester;

—OJC II 3/3: *cis* - 2,3 - dimethyl - 5,6 - dithiabicyclo 2.1.1 heptane 5-oxide (dithietane derivative).

Table 1. Inhibition of thromboxane B_2 biosynthesis

Fraction/compound	Thrombin concentration	TXB_2 content of PRP Supernatant (% of control) Concentration of the agent tested (mg/ml) 1.0	0.1	0.01	0.001	0.0 = Control (pg/ml)
LOI	1 I.U.	24	39	33	27	28,000
	10 I.U.	70	67	62	67	37,000
OJC	1 I.U.	9	25	47	26	28,000
	10 I.U.	11	20	43	72	37,000
OJC II	1 I.U.	14	4	12	25	28,000
	10 I.U.	61	32	9	29	37,000
OJC II 2	1 I.U.	5	6	41	n.d.	28,000
OJC II 3	1 I.U.	2	16	30	n.d.	28,000
OJC II 3/1 + 2	10 I.U.	5	16	55	91	15,882
OJC II 3/3	10 I.U.	9	34	89	102	15,882
Compound 1	10 I.U.	39	55	73	100	20,083
Compound 2	1 I.U.	16	44	72	90	8927
	10 I.U.	24	65	95	99	20,083

Inhibitory effects of ether extracted lyophilized onion juice (LOI), onion juice chloroform extracted (OJC), its subfractions and two synthetic thiosulfinates (Compound 1 and 2) on thrombin (1 and/or 10 I.U.)-stimulated thromboxane B_2 biosynthesis of human platelet-rich plasma (90,000 platelets/ml).

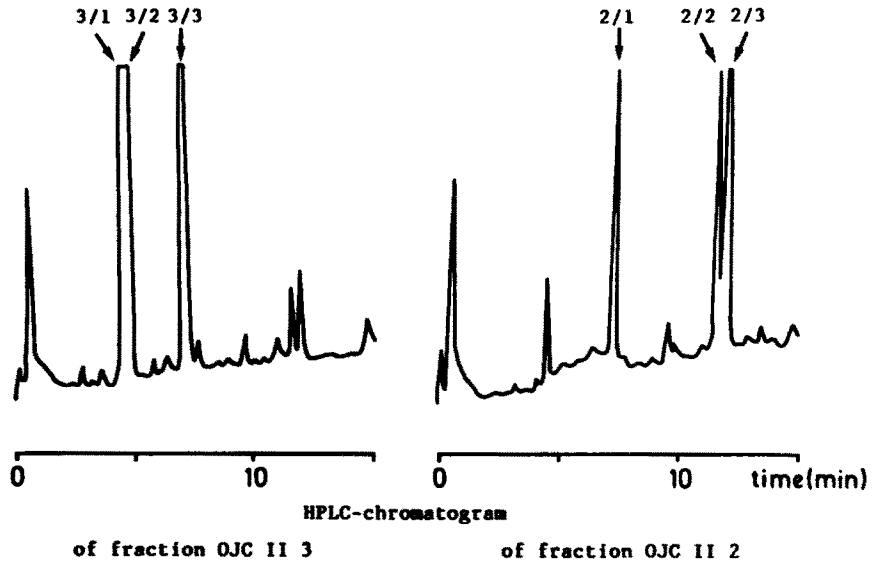


Fig. 4. HPLC chromatogram of fractions OJC II 3 and OJC II 2; stationary phase: LiCrospher 100 CH-18; column: Hibar, Merck; mobile phase: acetonitrile/water gradient system from 20% to 40% acetonitrile in 15 min; flow: 1 ml/min; detection at 200 nm.

Biological effects of alk(en)ylsulfinothioic acid alk(en)yl-esters and *dithietane derivatives*

Two types of compounds were identified in the most active fractions:

Alk(en)ylsulfinothioic acid alk(en)yl-esters. This group of compounds comprises OJC II 2/2, 2/3-1, 2/3-2, 3/1 and 3/2. The following experiments were performed with a mixture of compounds 3/1 and 3/2.

We observed:

—The activity of porcine 5-lipoxygenase was dose-dependently inhibited (Table 2: 100% from 5 μM concentrations onwards);

—Thromboxane B_2 biosynthesis of human platelets was suppressed dose-dependently up to more than 90% (Table 1).

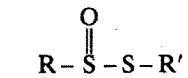
—PAF-induced bronchial obstruction of 7 guinea-pigs was markedly suppressed even by oral doses of 20 mg/kg (per cent inhibition: $40.8 \pm 20\%$, $P < 0.02$);

—Asthmatic reactions of 7 guinea-pigs due to allergen inhalation were reduced by 32.8% and 55.8% after oral treatment with 20 mg/kg OJC II 3 [1–2], Fig. 5, $P < 0.05$).

Dithietane derivatives. The dithietane derivatives, compounds OJC II 2/1 and OJC II 3/3, seem to exert no anti-asthmatic activity: OJC II 3/3 altered neither PAF- nor ovalbumin-induced bronchial obstruction in animals at doses of 20 mg/kg.

Biological effects of synthetic sulfinothioic acid-esters

The results reported above suggest that the protective activity of lipophilic onion extracts depends at least in part on the following chemical structure:



In order to test this hypothesis four different compounds were synthesized: dimethylthiosulfinate (compound 1); diphenylthiosulfinate (compound 2); di-(2-propenyl)-thiosulfinate (= Allicin; compound 3); dipropylthiosulfinate (compound 4).

These compounds exerted marked biological effects as follows.

Inhibition of 5-lipoxygenase in porcine leucocytes. The synthetic compounds 2 and 3 inhibited 5-lipoxygenases of porcine leucocytes even at the very low concentration of 2.5 μM (compound 2: 100%, compound 3: 27%). Compounds 1 and 4 did not show inhibitory effects up to 5 μM (Table 2).

Inhibition of leukotriene B_4 and C_4 biosynthesis of human peripheral leucocytes. In the human system we used higher concentrations (47 and 88 μM respectively. The synthetic compounds 2, 3 and 4 reduced markedly the biosyntheses of both leukotrienes B_4 and C_4 by human granulocytes after stimulation with calcium ionophore A 23187 'Table 3). Compounds 2 and 3 showed the best inhibitory effects. Compound 1 did not alter leukotriene biosynthesis in both concentrations.

Reduction of thromboxane biosynthesis. Compounds 1 and 2 reduced the thrombin-stimulated thromboxane B_2 biosynthesis of human platelets dose dependently up to 90% (Table 1).

Inhibition of histamine release from human peripheral leucocytes. 100 μM compound 3 (Allicin) as well as 10 and 100 μM compound 4 (dipropylthiosulfinate) significantly inhibited the anti-IgE induced histamine release from peripheral leucocytes of 11 atopic donors. All other compounds and concentrations showed no significant effects (Table 4). The release of histamine caused by the compounds themselves was below 5%.

Inhibition of PAF-induced bronchial obstruction in guinea-pigs. 50 mg/kg compound 1 given orally

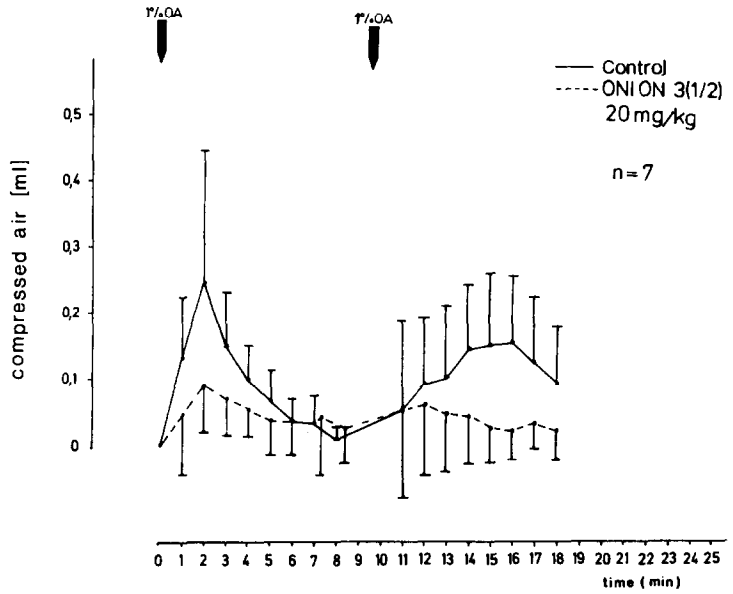


Fig. 5. Inhibition of PAF-induced bronchial obstruction of seven guinea-pigs by oral treatment with 20 mg/kg onion subfraction OJC II 3(1 + 2); $P < 0.001$ at time = 10 min, $P < 0.025$ at time = 20 min; per cent inhibition: 40.8 + 20%, $P < 0.02$.

45 min prior to the challenges abolished almost totally ($P < 0.005$) the asthmatic response of 10 guinea-pigs to the inhalation of PAF (Fig. 6).

These inhibitory effects were dose dependent: 25 mg/kg compound 2 achieved a per cent inhibition of 25.5 ± 21.3% ($P < 0.05$, $N = 9$), 50 mg/kg of 30.0 ± 15.4% ($P < 0.02$, $N = 8$) and 100 mg/kg of 52.9 ± 36.4% ($P < 0.01$, $N = 10$). Compounds 3 and 4 exerted insignificant effects ($P > 0.05$) up to doses of 50 mg/kg.

Reduction of allergen-induced bronchial obstruction in guinea-pigs. Allergen-induced bronchial asthma was inhibited by compounds 1 and 2. In comparison to the effects on PAF-induced reactions, the degree of inhibition, however, was less pronounced:

Fifty mg/kg compound 1 achieved a mean per cent inhibition of 30.4 and 26.7% ($P < 0.05$, $N = 10$), 100 mg/kg compound 2 a mean per cent inhibition of 50.8 and 20.6% ($P < 0.05$, $N = 10$). Compound 3 and 4 exerted insignificant effects up to doses of 50 mg/kg. Histamine- and acetylcholine-induced asthmatic reactions of each eight guinea pigs were not altered (data not shown).

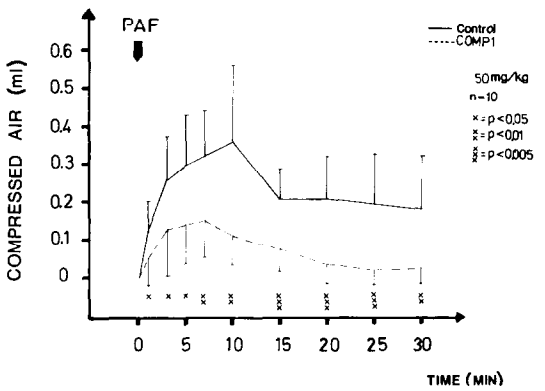


Fig. 6. Inhibition of PAF-induced bronchial obstruction in 10 guinea-pigs by 50 mg/kg compound 1 (= dimethylthiosulfinate).

DISCUSSION

In 1983 we reported [6] on the protective effects of onion extracts against allergen induced bronchial obstruction in human patients and experimental

Table 2. Inhibition of 5-lipoxygenase

Compound	Per cent inhibition of porcine 5-LO by 1 μM	2.5 μM	5 μM
1	0	0	0
2	76	100	100
3	n.d.	27	85
4	0	0	0
3 (1 + 2)	24	40	100

Inhibitory effects of isolated (compounds 3 (1 + 2)) and synthetic (compounds 1–4) thiosulfinates on 5-lipoxygenase activity in porcine leucocytes.

animals. Furthermore, the inflammatory reactions of human skin were suppressed by topical treatment with onions and PAF-induced bronchial obstruction of guinea pigs was counteracted by oral pretreatment with lyophilized onion juice [7, 9, 17].

Since dithietanes (compounds OJC II 2/1 and OJC II 3/3) isolated from the active fractions showed no protective activity, we assume that thiosulfinates represent the main anti-asthmatic principle of onions. We were, however, unable to recover the total activity of the chloroform extract. Maybe other anti-asthmatic thiosulfinates have not been isolated as yet or thiosulfinates have been degraded to inactive disulfides or thiosulfonates during fractionation. Other compounds formerly found in onion extracts such as adenosin [18], alliin [19], cycloalliin [20], the flavonoid quercetin [21] or mustard oils [7, 22] can be excluded as the main anti-asthmatic principle since they have not been found in the active lipophilic extract.

The mode of action of thiosulfinates cannot be attributed to one single effect:

In vitro we observed inhibition of histamine release, lipoxygenase activity, leukotriene and thromboxane biosynthesis (this paper) as well as non-toxic inhibition of cell functions as chemotaxis and PAF-induced oxidative burst (to be published). The most prominent *in vivo* effect investigated as yet was inhibition of PAF-induced as well as allergen-dependent bronchial obstruction. Pulmonary reactions to inhaled histamine or acetylcholine were not altered, therefore a direct bronchodilatory effect of thiosulfinates seems unlikely.

In an attempt to study the structure–activity relationship of thiosulfinates, dimethyl-, diallyl-, dipropyl- and diphenylthiosulfinate were synthesized. Synthetic thiosulfinates exhibited comparable *in vitro* and *in vivo* activities. This indicates that the –S(O)–S–moiety is the active center of the molecule. The extent of the biological activities as well as the stability of thiosulfinates are markedly affected by the side chains. Allicin did not show significant anti-asthmatic effects *in vivo*, most likely because of its instability. Asymmetrically substituted and partly unsaturated thiosulfinates as identified in onions were more active than symmetrically substituted compounds in inhibiting PAF- and allergen-induced bronchial obstruction *in vivo* as well as thromboxane biosynthesis *in vitro*. In contrast, diphenylthiosulfinate is the most potent 5-lipoxygenase inhibitor found in this investigation and only diallyl- and dipropylthiosulfinate significantly reduced histamine release from human leucocytes. We assume that the substitution pattern on either side of the thiosulfinate moiety decides which mode of thiosulfinate action predominates, e.g. inhibiting leukotriene or thromboxane biosynthesis, histamine release or counteracting PAF-induced effects.

Acknowledgements—The authors thank B. Höckmeier, E. Luxemburger, R. Mayer, S. Vollmer and S. Walther for their skillful technical assistance. We gratefully acknowledge financial support of this work by the Deutsche Forschungsgemeinschaft (DFG).

Note added in proof: After completing the manuscript, we found other new sulfur-containing compounds in onion extracts: six different sulfinyldisulfides inhibited both lipoxygenase and cyclooxygenase by more than 50% at 1 μM concentrations (Th. Bayer: Neue schwefelhaltige Inhaltsstoffe aus Allium cepa L. mit antiasthmatischer und antiallergischer Wirkung, Thesis in Pharmacy and Chemistry, University of Munich, June 1988). Kawakishi and Morimitsu described one similar compound as "new inhibitor of platelet aggregation in onion oil" (*Lancet*, August 1988, 330).

REFERENCES

1. Joachim H, *Papyros Ebers*. Georg Reimer Verlag, Berlin, 1890.
2. Korting GW and Brehm G, *Dermatologische Notfälle in der Klinik und Praxis*, p. 16. Georg Thieme Verlag, Stuttgart, F.R.G. 1967.
3. Vanderhoek JY, Makheja AN and Bailey JM, Inhibition of fatty acid oxygenases by onion and garlic oils. *Biochem Pharmacol* **29**: 3169–3173, 1980.
4. Apitz-Castro R, Cabrera S, Cruz MR, Ledezma E and Jain MK, Effects of garlic extract and of three pure components isolated from it on human platelet aggregation, arachidonate metabolism, release reaction and platelet ultrastructure. *Thromb Res* **32**: 155–169, 1983.
5. Baghurst KJ, Raj MJ and Truswell AS, Onions and platelet aggregation. *Lancet* **i**: 101, 1977.
6. Dorsch W, Adam O and Weber J, Antiallergic and antiasthmatic effects of onion extracts. European Academy of Allergy and Applied Immunology, abstract in: *Folia Allergol Immunol Clin* **30**: (suppl. to No. 4): 17, 1983.
7. Dorsch W, Adam O, Weber J and Ziegeltrum T, Antiasthmatic effects of onion extracts—detection of benzyl- and other isothiocyanates (mustard oils) as antiasthmatic compounds of plant origin. *Eur J Pharmacol* **107**: 17–23, 1985.
8. Dorsch W and Ring J, Suppression of immediate and late anti-IgE-induced skin reactions by topically applied alcoholic onion extracts. *Allergy* **39**: 43–49, 1984.
9. Dorsch W, Ettl M, Hein G, Scheftner P, Weber J, Bayer T and Wagner H. Antiasthmatic effects of onions. *Int Archs Allergy Appl Immun* **82**: 535–536, 1987.
10. Small LD, Bailey JH and Cavallito CJ, Alkyl thiolsulfinates. *J Am Chem Soc* **69**: 1710–1713, 1947.
11. Kuhl P, Shiloh H, Jha H, Murawski U and Zilliken F, 6,7,4′-Trihydroxyisoflavan: A potent and selective inhibitor of 5-lipoxygenase in human and porcine peripheral blood leukocytes. *Prostaglandins* **28**: 783, 1984.
12. Hjorth V, Jansson AK and Vretblad P, Purification of human neutrophil granulocytes. *J Immunol Methods* **43**: 95–101, 1981.
13. Strasser T, Fischer S and Weber PC, Leukotriene B_5 is formed in human neutrophils after dietary eicosapentaenoic acid. *Proc Natl Acad Sci USA* **82**: 1540, 1985.
14. Siess W, Roth P and Weber PC, Stimulated platelet aggregation, thromboxane B_2 formation and platelet sensitivity to prostacyclin: a critical evaluation. *Thromb Haemost* **45**: 204–209, 1981.
15. Ring J and O'Connor R, *In vitro* histamine and serotonin release studies in atopic dermatitis. *Int Arch Allergy Appl Immun* **58**: 322–330, 1979.
16. Dorsch W, Waldherr U and Rosmanith J, Continuous recording to intrapulmonary "Compressed Air" as a sensitive noninvasive method of measuring bronchial obstruction in guinea pigs. *Pflügers Arch* **391**: 236–241, 1981.
17. Dorsch W, Adelmann-Grill B, Bayer T, Ettl M, Hein G, Jaggy H, Ring J, Scheftner P and Wagner H, Zwiebelextrakte als Asthma-Therapeutika? *Allergologie* **10**: 316–324, 1987.
18. Weisenberger H, Grube H, Koenig E and Pelzer H,

Isolation and identification of the platelet aggregation inhibitor present in the onion, Allium cepa. *FEBS Lett* **26***(1)*: 105–108, 1972.

19. Liakopoulou-Kyriakides M, Sinakos Z and Kyriakides DA, Identification of alliin, a constituent of *Allium cepa* with an inhibitory effect on platelet aggregation. *Phytochemistry* **24**: 600–601, 1985.
20. Augusti T, Benaim ME, Dewar HA and Virden R, Partial identification of the fibrinolytic activators in onion. *Atherosclerosis* **21**: 409–416, 1975.
21. Herrmann K, Uber die Flavonole und Phenole der Zwiebel (*Allium cepa* L.). *Arch Pharm (Weinheim)* **291**: 238–247, 1958.
22. Hanley AB, personal communication (1986).