than that of spirographis porphyrin dimethyl ester, and therefore can be easily distinguished from spirographis porphyrin. The properties of the two isomers of monoformylmonovinyl porphyrins are similar to those reported by Inhoffen *et al.* (1966, 1969).

The biological and biophysical properties of the iron complexes of these formylporphyrins after recombination with human apohemoglobin will be shown elsewhere (Asakura and Sono, 1974).

Acknowledgment

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The Isolation and Characterization of γ-L-Glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine Sulfoxide from Sandal (*Santalum album* L). An Interesting Occurrence of Sulfoxide Diastereoisomers in Nature†

Ramadasan Kuttan, N. Gopalan Nair, A. N. Radhakrishnan, T. F. Spande, H. J. Yeh, and B. Witkop*

ABSTRACT: γ-L-Glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine sulfoxide (**1**) has been isolated from sandal (*Santal album* L.) where it comprises approximately 0.5% of the weight of the dried leaves. The structure was proved by nuclear magnetic resonance, ir, and circular dichroism spectroscopy, by acid and enzymatic hydrolyses and by comparison with a sample of **1** previously isolated from onion (*Allium cepa*). Circular dichroism measurements established that the sulfoxide group in the sandal and onion peptides are of opposite configurations.

A routine amino acid analysis of sandal (*Santalum album* L.) leaves by two-dimensional chromatography revealed two unknown spots. One was identified as the polyamine, *sym*-homospermidine (Kuttan *et al.*, 1971). Material from the other spot, when examined with the amino acid analyzer, revealed a peak in the region of the acidic amino acids, emerging 15 min before *trans*-4-hydroxyproline. This acidity was exploited in isolating this compound by ion-exchange chromatography. Acid and enzymatic hydrolyses combined with proton magnetic resonance (pmr), circular dichroism (CD), and ir spectroscopy established the structure of the unknown as γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine sulfoxide (**1**).

The sulfoxide diastereoisomer of **1** had previously been isolated from onion (*Allium cepa*) by Virtanen and Matik-

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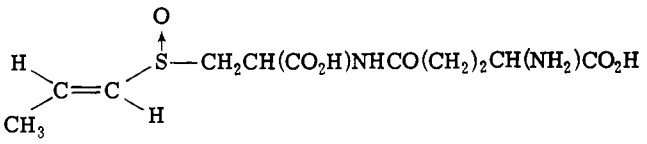
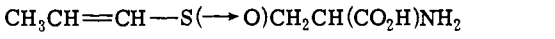


1

kala (1961b,c) who showed that cleavage of the glutamyl peptide bond with a beef kidney preparation yielded the so-called lacrimatory precursor (**2**), also found in onion. When **2** was exposed to an enzyme released from crushed onion, pyruvate, ammonia, and the onion lacrimatory factor (**3**) resulted.



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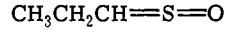

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In the following studies the peptide isolated from sandal is completely characterized and compared with the peptide isolated from onion.

Experimental Section

Materials

Chemicals. The following were obtained as indicated: Dowex 50-X8, H^+, beads, reagent (J. T. Baker); fluoro-2,4-dinitrobenzene (Eastman Kodak Co.); 5,5′-dithiobis(2-nitrobenzoic acid) (Sigma Chemical Co.); *N*-ethylmaleimide (Schwarz Bioresearch); samples of **1** and **2,** isolated from onion, were gifts of Professor A. I. Virtanen (see acknowledgment). All other reagents or solvents used were of analytical or the best available commercial grade.

Instruments. Infrared spectra in KBr discs were obtained with a Perkin-Elmer Model 421 spectrophotometer. Circular dichroism spectra were measured with 0.1–0.2% aqueous solutions of **1** in 0.1-cm cells with a Cary Model 60 spectropolarimeter. Ultraviolet spectra were recorded with a Cary Model 11 spectrophotometer and 1-cm cells. Proton magnetic resonance spectra were obtained in D_2O containing TSP[1] or DSS as internal standards with a Varian Associate HA-100 spectrometer. Chemical shifts are expressed in δ values (ppm) relative to the standard.

Methods

Chromatography. Two-dimensional paper chromatography was performed according to Subramanian and Rao (1955) on Whatman No. 1 paper. Solvent systems used were A, phenol–KCl–HCl buffer (pH 1.0) (50:7 v/v), in the first direction and B, 1-butanol–acetic acid–water (4:1:1) in the second. One-dimensional paper chromatography was done in B, unless otherwise stated. Spots were visualized by spraying with ninhydrin (0.4% in acetone containing 2% 2,4,6-collidine) and by heating at 60° for 10 min. Thin (0.25 mm) layers of silica (F-254 Merck, Darmstadt) and "Avicel" cellulose (Analtech, Wilmington) were developed with B or a 5:2:4 mixture (C), and spots were detected with the uv hand lamp (silica) or a ninhydrin spray (0.1% in methanol–1-butanol–2 N acetic acid (20:10:1)) and brief heating at 100° (silica or cellulose).

Amino acid analyses were carried out according to the method of Benson and Patterson (1965) with the automatic amino acid analyzer (Woods-Jerenberg model) and Beckman custom research resins PA 28 and PA 35.

N-Terminal analysis was done according to the method of Sanger (1945) with fluoro-2,4-dinitrobenzene.

Performic acid oxidation was performed according to the method of Moore (1963).

Hydrolyses were carried out at 100° in sealed tubes with 6 N HCl for 18 hr (complete) or 1 N HCl for 2 hr (partial).

Enzymatic cleavage of **1** was effected with a monkey kidney homogenate prepared following the procedure of Orlowski and Meister (1970) for obtaining γ-glutamyl transferase (EC 2.3.2.1) from beef kidney. Monkey kidney (5 g) was homogenized in the cold with 15 ml of 0.08 M $MgCl_2$ (pH 9.0). An incubation mixture consisting of compound **1** (5 mM) from sandal, $MgCl_2$ (11 mM), Tris-HCl (pH 9.0) (100 mM), and monkey kidney homogenate (0.2 ml) in a total volume of 0.5 ml was kept at 37° for 4 hr. The reaction was then stopped by the addition of 3 ml of ethanol and the mixture centrifuged. The aqueous layer containing the amino acids was separated by the addition of 6 ml of chloroform.

Enzymatic conversion of **2** *to* **3** was achieved by an enzyme preparation from onion (Spare and Virtanen, 1963). Onions (10 g) were homogenized with 30 ml of water in the cold. Endogenous **2** was removed by dialysis against Tris buffer (0.01 M, pH 8.5) and 0.2 ml of this preparation was added to the above hydrolysate.

Isolation. EXTRACTION. Sandal leaves, dried at 50°, were powdered and passed through a 40-mesh sieve; 2.3 kg of powder was extracted by percolation in five batches in a home made extractor with 14 l. of boiling water. The pH of the extract (10 l.) was adjusted to pH 1.5, toluene was added as a preservative, and the extract was allowed to stand overnight at 4°. After removal of a fine precipitate by centrifugation (10^4 g, 10 min), the clear brown supernatant was processed by the ion-exchange procedure below.

FIRST DOWEX 50 COLUMN. The extract was passed through a 4.5 × 130 cm Dowex 50 (H^+ form) column and 500-ml fractions were collected which were monitored for amino acids by the use of paper chromatography and solvent system B. Compound **1,** accompanied by *cis*-4-hydroxyproline, glutamic acid, and aspartic acid, appeared in fraction 17 and thereafter. When the entire extract had passed through the column (fraction 20), the column was washed with 14 l. of water. Fractions 16–21, highly colored, containing **1** and the acidic amino acids were pooled (fraction 1a) while fractions 22–23, light brown, containing mainly **1,** were pooled (fraction 1b), concentrated *in vacuo,* and processed as described below.

SECOND DOWEX 50 COLUMN. Fraction 1b (50 ml) was adjusted to pH 1.0 and passed through a 2.2 × 50 cm Dowex 50 column (H^+ form). After 1 l. of water was passed through the column, 0.25 N ammonium hydroxide was used as eluent and 20-ml fractions were collected. Compound **1** was present, chromatographically pure in fractions 51–63 (pooled fraction 2a). Fractions 64–72 contained **1** contaminated by a few other amino acids (pooled fraction 2b). Fractions 1a and 2b were combined and rechromatographed on a 1-l. Dowex column by the above procedure to obtain another batch of pure **1.**

FURTHER PURIFICATION. The combined batches of **1** were concentrated to 50 ml and 1 l. of acetone was added. The acetone layer was decanted and the precipitated, sticky material was redissolved in 50 ml of water and reprecipitat-

[1] Abbreviations used are: TSP, sodium 3-(trimethylsilyl)propionate-d_4; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate-d_6.

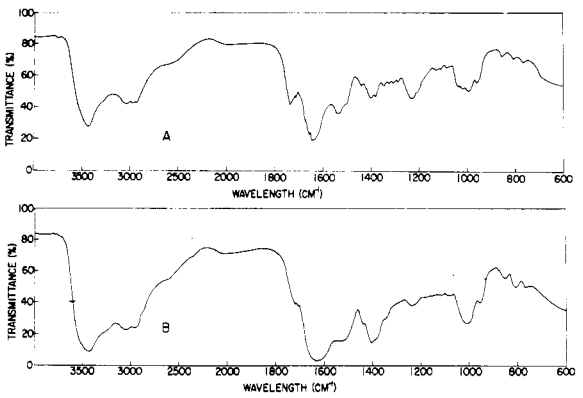


FIGURE 1: Infrared spectra of γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine sulfoxide (**1**) from sandal leaves (A) and onion (B). Concentration *ca.* 0.6 mg in 300 mg of KBr.

ed with acetone. This procedure was repeated once more. The precipitate was filtered, washed with 90% acetone, and dried *in vacuo* over P_2O_5 to yield 3.4 g of light brown powder.

CRYSTALLIZATION. To a solution of the above material in 15 ml of water and 100 ml of absolute ethanol was added 200 mg of activated charcoal and the slurry shaken well for 15 min and filtered. The filtrate was heated on a water bath while acetone was added dropwise until a slight turbidity resulted. On cooling to room temperature, then to 4° overnight, a crop of 2.5 g of colorless, granular crystals resulted. This material was recrystallized from aqueous acetone and dried *in vacuo* over P_2O_5 to afford 1.5 g of crystals.

QUANTITATION IN LEAVES. A number of sandal trees near the Wellcome Research Unit were examined for the presence of compound **1**. The leaves from young plants contained only traces while much larger amounts were found in older plants. The amino acid analyzer permitted a quantitative estimation of the peptide in the plant leaves from which the peptide was isolated. Here the peptide constituted nearly 0.5% of the dried leaves. Fresh onions contain nearly 0.2% **1** by weight (Matikkala and Virtanen, 1967), while dehydrated onions contained 0.15% of **2** (Carson *et al.*, 1966).

Results

Physical properties: colorless, granular crystals, mp 140° dec; $[\alpha]^{20}D$ −37.1° (*c*, 0.9%, H_2O). *Anal.* Calcd for $C_{11}H_{18}N_2SO_6 \cdot H_2O$: C, 40.70; H, 6.18; N, 8.65; S, 9.89. Found: C, 41.41; H, 6.05; N, 8.55; S, 9.80.

Spectral Characterization. UV ABSORPTION. Compound **1** did not show any characteristic absorption peak, although a very weak, broad peak centered at 295 nm (ϵ 67) and a just perceptible shoulder at 230 nm (ϵ 2.15 × 10^3) could be observed in water.

IR ABSORPTION. As seen in Figure 1, stretching vibrations for the carbonyl group of a carboxylic acid (1730 cm^{-1}) and an amide (1650, 1530 cm^{-1}), overlapping with a carboxylate carbonyl absorption at 1610 cm^{-1}, are evident. The moderately strong absorptions at 1010 and 955 cm^{-1} may be ascribed to the unsaturated sulfoxide group and a trans-substituted double bond, respectively (Nakanishi, 1962). Absorptions at *ca.* 2500 probably arise from NH_3^+ and the carboxyl O–H stretching frequencies while bands at 3420–2920 (NH_3^+) and 1230 cm^{-1} (C–N stretching?) may arise from an amine function. Also shown on Figure 1 for purposes of comparison is the spectrum of the peptide from onion supplied by Virtanen. Differences in peak intensities may be due to diastereoisomerism or variations in hydration. The onion peptide is extremely hygroscopic. While the elemental analysis of the sandal peptide fits a monohydrate, it did not appear to be particularly hygroscopic.

CD ABSORPTION. Between 280 and 205 nm, a single *negative* maximum was observed for an aqueous solution of the sandal peptide. The molar ellipticity [ϕ] at this wavelength (237.5 nm) was -1.36×10^4 ($\Delta\epsilon = -4.12$). The onion peptide in this spectral region exhibited a single *positive* maximum at 237.5 nm, [ϕ] = $+1.58 \times 10^4$ ($\Delta\epsilon$ = +4.79).

PMR SPECTRA. The sandal peptide in D_2O (DSS) showed the following peaks at 100 MHz: quartet (J = 5.8 Hz) of doublets at 6.71 (CH_3CH= (B)), doublet at 6.52 (=CHS→O (A)), total of two protons, collapsing to an AB quartet centered at 6.61 ($|J_{AB}|$ = 15.2 Hz) on irradiation at center (1.93) of 3-proton allylic methyl doublet, J_{B,CH_3} = 5.8 Hz; a one-proton triplet (J = 6 Hz) centered at 3.90 (Glu α-H), a two proton eight-line multiplet representing the AB (CH_2—S→O) portion of an ABX pattern with δ_B 3.48, δ_A 3.28, J_{AX} = 10, J_{BX} = 4, and J_{AB} = 14 Hz revealed by decoupling the Cys α-H proton (X) by irradiation at the center of a quartet largely hidden by the HOD peak at 4.70; a two-proton multiplet centered at 2.54 (a perturbed triplet, glutamyl CH_2CONH—) and a two-proton broadened triplet at 2.20 (glutamyl β protons; coupled to adjacent glutamyl α-H, $J \simeq 6$ Hz). A nearly identical spectrum resulted for the onion peptide (TSP) with the exceptions that signals for the α protons of Cys and Glu were moved upfield to 4.45 and 3.78, respectively. When the pH of both samples was adjusted to 7.2 (1 N NaOD), this discrepancy disappeared and these signals appeared in both spectra at the positions noted for the onion peptide.

Sodium metabisulfite (100 mg) was added directly to the sandal peptide (44 mg) nmr tube; the tube was shaken well and allowed to stand at 45° for 90 hr. On rescanning, the vinyl signals had shifted upfield and interchanged so that the doublet (J = 15 Hz) appeared at 6.00 while the octet was centered at 5.80. In addition the allylic methyl doublet (J = 5.0) had shifted upfield from 1.93 to 1.70. The bisulfite reduction was 92% complete as judged by the 1.93 doublet (*ca.* 8%) from residual starting material. The eight-line pattern for the cysteinyl methylene signals had also shifted upfield to δ_B 3.24 (quartet) and δ_A 3.04 (quartet) (J_{BX} and J_{AX} remained the same). Signals for the α protons for Cys (4.54 quartet) and Glu (3.92 triplet) as well as the methylene groups of Glu (2.52 and 2.21) were virtually unchanged.

A sample of *S*-(1-propenyl)cysteine sulfoxide (**2**) donated by Professor Virtanen was examined in D_2O (TSP) and the following signals were recorded: octet at 6.78 (=C*H*CH_3, J_{H,CH_3} = 5.5 Hz), doublet at 6.52 (=CHS→O, J = 15 Hz); an AB quartet with δ_B 6.70, δ_A 6.53. J_{AB} = 15.2 Hz resulted from these signals when the center of the allylic methyl doublet (1.95) was irradiated. An eight-line AB portion of an ABX pattern appeared with quartets centered at 3.46 (B) and 3.27 (A) (J_{AX} = 8, J_{BX} = 5.5, and J_{AB} = 14 Hz) and is ascribed to the cysteine methylene group. The α proton (X) gave rise to a quartet at 4.10.

Chemical Properties. Two-dimensional chromatograms of complete hydrolysates of **1** revealed only glutamic acid and cystine. The amino acid analyzer confirmed this and indicated their proportion to be 2:1, respectively. In addition

cystine was identified by its oxidation to cysteic acid as well as reduction to cysteine and reaction with sodium nitroprusside.

Partial hydrolysis gave only glutamic acid. Further hydrolysis (6 N HCl, 18 hr) gave no cystine nor was there any change in the concentration of glutamic acid indicating that partial hydrolysis had completely liberated the glutamic acid and suggesting a γ-glutamyl peptide. The N-terminal was found to be glutamic acid by the fluoro-2,4-dinitrobenzene method.

Performic acid oxidation directly upon **1** produced no cysteic acid; however, cysteic acid did result in oxidation of a complete hydrolysate of the peptide. Compound **1** did not react with sulfhydryl reagents, such as nitroprusside,. 5,5′-dithiobis(2-nitrobenzoic acid), *N*-ethylmaleimide, or ammonium phosphomolybdate, indicating the absence of a free -SH group. Disulfide groups were also absent, since mild reduction produced no detectable -SH groups.

Cystine and glutamic acid isolated from a 6 N HCl hydrolysate were both of the L configuration as indicated by the specific rotation[2] of the former and the observation that the latter reduces NAD^+ in the presence of monkey liver dehydrogenase (Strecker, 1955).

An enzyme preparation from monkey kidney cleaved compound **1** to glutamic acid and the lacrimatory precursor (**2**). The reaction products were identified by paper chromatography in solvent system A in which one component had an R_F (0.16) identical with glutamic acid, while the other had an R_F (0.63) identical with that of **2** obtained from onion. The kidney enzyme hydrolysate on treatment with the onion enzyme yielded pyruvic acid and the lacrimator **3,** identified by reaction with 2,4-dinitrophenylhydrazine (Friedemann, 1957) and a pronounced lacrimatory effect (Spare and Virtanen, 1963), respectively. The lacrimatory effect was checked with a number of volunteers from the Wellcome Research Unit and in each case there was unequivocal evidence for lacrimation compared to control tubes without **1.**

Discussion

The γ-L-glutamyl peptide (**1**) of *S*-(1-propenyl)-L-cysteine sulfoxide (**2**) is the principal γ-glutamyl peptide[3] of onion (*Allium cepa*) (Virtanen and Matikkala, 1961b,c) being accompanied by lesser amounts of γ-glutamyl-*S*-(2-carboxypropyl)cysteine (**4**) (Matikkala and Virtanen, 1970) and -*S*-methylcysteine (Virtanen and Matikkala, 1961a) among others (Virtanen and Matikkala, 1960a,b, 1961a; Matikkala and Virtanen, 1967). γ-Glutamyl peptides of S-alkylated cysteine are also found in garlic (*Allium sativum*) and chives (*Allium shoenoprasum*) and, while related to **1,** exhibit interesting structural differences. Thus chives contain **5,** the reduced, thioether analog of **1** (Virtanen and Matikkala, 1962; Matikkala and Virtanen, 1962), as well as γ-glutamyl-*S*-propylcysteine (Matikkala and Virtanen, 1963), while garlic contains the latter (Virtanen *et al.,* 1962) accompanied by γ-glutamyl-*S*-allylcysteine (Virtanen and Mattila, 1961a).

$RSCH_2CH(CO_2H)NHCO(CH_2)_2CH(NH_2)CO_2H$

4, R = $CH_3CH(CO_2H)CH_2$

5, R = $CH_3CH{=}CH$

Simple cysteine derivatives, *e.g.,* *S*-methyl-, *S*-propyl-, and *S*-(1-propenyl)cysteine, occur in onion in both the thioether and sulfoxide form, the latter in garlic in the sulfoxide form (Sugii *et al.,* 1963), and *S*-allylcysteine as the thioether in onion and the sulfoxide in garlic,[4] *but the γ-glutamyl peptides of these cysteine derivatives are rarely found in the oxidized state* (Virtanen, 1965). The single exception seems to be the occurrence in lima beans of γ-glutamyl-*S*-methylcysteine sulfoxide as a minor component accompanying the unoxidized peptide (Rinderknecht, 1957, 1958). This same sulfoxide which is also found in small amounts with γ-glutamyl-*S*-methylcysteine in red kidney bean extracts may be an artifact arising on air oxidation either during the extraction or during paper chromatography (Zacharius *et al.,* 1959). Virtanen and Matikkala ruled out (1961c) the possibility that **1** was an oxidative artifact of **5** by showing that **2** prepared from **1** by action of a beef kidney enzyme has the same rotation and hence the same sulfoxide configuration as **2** isolated crystalline from onion. Our circular dichroism spectrum of **1** from onion corroborates a highly stereospecific and presumably enzymatic oxidation of some precursor (*e.g.,* **5** or *S*-(1-propenyl)cysteine) in onions. Likewise, we conclude that the sandal peptide **1** originates by a stereospecific oxidation, in this case, to a sulfoxide configuration opposite to that of the onion peptide.

For our structure proof, we relied upon chemical hydrolysis to glutamic acid and cystine. Milder acid hydrolysis liberated glutamic acid and indicated the presence of a γ-glutamyl linkage in the peptide, a peptide in which glutamic acid was also N-terminal as indicated by the DNP assay. At this point, spectroscopic data indicated that the peptide had structure **1.** Enzymatic hydrolysis with a monkey kidney preparation, then exposure of this hydrolysate to an onion enzyme extract, liberated a lacrimator, presumably **3.**[5] Fi-

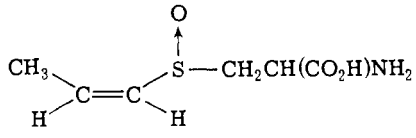
[2] A cystine sample obtained by combined preparative paper (solvent B) and ion-exchange chromatography was contaminated by a ninhydrin-negative, positive-rotating impurity. The observed rotation $[\alpha]^{20}D$ −63° (*c* 1.8, 5 N HCl), however, supports the L configuration (lit. −232° (Meister, 1965)). Pmr (D_2O) and ir (KBr) spectra indicated the sample to be substantially cystine. The presence of appreciable *meso*-cystine is ruled out by the automatic amino acid analyzer.

[3] Two reviews (Fowden, 1964; Waley, 1966) discuss the occurrence of γ-glutamyl peptides in plants.

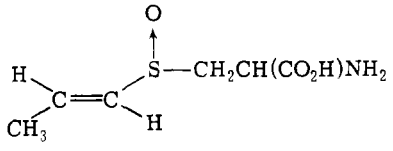
[4] This sulfoxide (alliin) was isolated by Stoll and Seebeck (1948, 1949) and shown to be the precursor of the potent bactericide allicin ($CH_2{=}CHCH_2S(\rightarrow O)SCH_2CH{=}CH_2$) under the action of garlic alliinase. Alliin with a dialyzed onion extract does not generate a lacrimator (Spare and Virtanen, 1963, Virtanen, 1965). Alliinase from either garlic (Stoll and Seebeck, 1951) or onion (Schwimmer and Mazelis, 1963) shows the same preference for (+) over (−) diastereoisomers of L-cysteine sulfoxide as exhibited by the C-S lyases (*Albizzia lophanta,* Schwimmer and Kjaer, 1960; *Allium cepa,* Schwimmer *et al.,* 1964; broccoli, Mazelis, 1963) which have been studied, although the latter have much reduced substrate specificity. In view of the fact that **1** from onion and sandal are of opposite configuration at the sulfoxide group, the derived lacrimatory precursors (**2**) must also be diastereoisomers. At this time we have not yet studied the kinetics for the production of **3** from **2** (sandal) using the onion enzyme.

[5] The propenylsulfinic acid structure $CH_3CH{=}CHSH\rightarrow O$ was originally proposed for **3** by Virtanen and Spare (Virtanen and Spare, 1962; Moisio *et al.,* 1962; Spare and Virtanen, 1963). This has been revised to the thiopropanal oxide structure, a sulfine, as a result of work by Wilkens (1961) and Brodnitz and Pascale (1971). Consistent with this structure is the observation of Carson and coworkers that either of the sulfoxide diastereoisomers (**2a**) from synthetic *S*-(*cis*-1-propenyl)-L-cysteine produces a lacrimator when exposed to an onion enzyme preparation (Carson and Wong, 1963; Carson and Boggs, 1966). It should also be mentioned that geometric isomers are possible for **3** (*i.e.,* **3a, 3b**) (*cf.* King and Durst, 1966; Tangerman and Zwanenburg, 1973). Consequently, unless a rapid room temperature equilibration intervenes, one sulfoxide diastereoisomer of either **2a** or **2b** should generate cis lacrimator **3a** while the other diastereoisomers should produce the trans isomer **3b.**

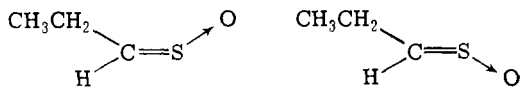
nally the onion and sandal peptides had identical R_F's on thin layers of cellulose (0.16, solvent B; 0.54, solvent C) or silica (0.21, solvent C).



2a



2b

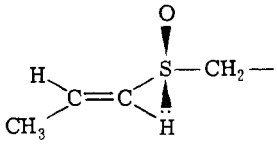


3a **3b**

Summary of the Spectral Data. The proton magnetic resonance spectrum of **1** from sandal provides unambiguous evidence that the alkyl group attached to cysteine is an *S*-(1-propenyl) group with trans configuration. An allylic methyl group (δ 1.93) coupled (J = 5.8 Hz) to an adjacent vinyl proton (δ 6.71) which is in turn coupled to a second vinyl proton (δ 6.52, CHS→O) was observed. The coupling constant (J = 15 Hz) of this latter interaction strongly supports a trans orientation of the two vinyl protons (*cf.* Carson *et al.*, 1966). The sizable upfield shifts for the vinyl quartet ($\Delta\delta$ = 0.91), the vinyl doublet ($\Delta\delta$ = 0.52), the cysteine methylene group ($\Delta\delta$ = *ca.* 0.30), and the allylic methyl ($\Delta\delta$ = 0.23)—all signals from protons in close proximity to the cysteine sulfur atom—on treatment with bisulfite, a reagent known to effect the reduction of sulfoxides (Micheel and Schmitz, 1939), support a sulfoxide group in **1**. The vinyl proton (δ 6.67) adjacent to the sulfoxide group in methyl vinyl sulfoxide shifts upfield by 0.25 ppm on reduction of the sulfoxide group (Chapman and Magnus, 1966). The trans-substituted double bond and the sulfoxide group derive further support from the moderately strong ir absorptions observed at 955 and 1010 cm^{-1}, respectively (*cf.* Spare and Virtanen, 1963; Zacharius *et al.*, 1959). Finally the good correspondence between the negative CD maximum at 237.5 nm, a shoulder observed at approximately 230 nm in the uv, and Karrer and coworkers' report (1951) on the uv absorption of $C_{12}H_{25}S(\rightarrow O)CH{=}CH_2$ ($\lambda_{sh}^{H_2O}$ 230 nm; ϵ 2.8 × 10^3) and the natural product sulforaphen, $CH_3S(\rightarrow O)CH{=}CH{-}(CH_2)_2N{=}C{=}S$ (λ_{max}^{EtOH} 231 nm; ϵ 3.24 × 10^3) provide additional evidence for a sulfoxide grouping with an adjacent unsaturated group. A saturated sulfoxide would absorb at somewhat shorter wavelengths (*ca.* 190–210 nm; Mislow *et al.*, 1965).

Virtanen and Spare, in establishing the structure of **2** (Virtanen and Spare, 1962; Moisio *et al.*, 1962; Spare and Virtanen, 1963), left unresolved the configuration of the propenyl group. Recently Carson and coworkers have shown (Carson *et al.*, 1966) by pmr that the configuration of **2** which they isolate from onion is clearly trans (*i.e.*, **2b**). We have examined by pmr samples of **1** and **2** isolated by the Helsinki group from onion as well as **1** isolated from sandal and have found (see Results section) that all possess a trans propenyl group. In addition samples of **1** from either onion or sandal had virtually identical pmr spectra; in this instance diastereoisomers cannot be clearly differentiated by pmr.

The oxidation of the presumed precursor **5** to give **1** in onion or sandal is apparently stereospecific as judged by the positive and negative maxima observed in their respective circular dichroism spectra. One of the two possible sulfoxide configurations must be the sole or predominant product in either case since the absolute values of the molar ellipticities are approximately the same. Such stereospecificity is apparently not usual in the onion, however, for racemic sulfoxide mixtures occur in the case of *S*-methyl- and *S*-propyl-L-cysteine and L-methionine (Matikkala and Virtanen, 1967). If the same assignments (Mislow *et al.*, 1965) obtain for both *S*-propenyl and for methyl alkyl sulfoxides, a positive CD maximum at 237.5 nm would indicate an *S* configuration for the onion sulfoxide group, while a negative maximum at that wavelength would correspond to an *R* configuration for the sandal sulfoxide group. The *R* configuration is shown below (**1a**) for the sandal peptide. Mislow



1a

and coworkers (Axelrod *et al.*, 1968a,b) have cautioned on extending a rule worked out for simpler sulfoxides to systems where a sulfoxide substituent could perturb the sulfoxide n→d transition.[6] Such would certainly be the case for the unsaturated *S*-propenyl group; consequently, the above assignments must be regarded as provisional. Hermann and coworkers (1971) have shown that the positive and negative CD maxima at approximately 220 nm which result from the two *N*$^{\epsilon}$-acetyl-L-thialysine sulfoxide diastereoisomers correspond to the *S* and *R* configurations, respectively, of the sulfoxide group and that amino acid and amide carbonyl absorptions can be ignored. A similar conclusion had been reached earlier by Gaffield and coworkers (1965) with regard to the L-amino acid contributions to the positive and negative ORD curves of *S*-alkyl-L-cysteine sulfoxide diastereoisomers.

Interestingly most of the optically active sulfoxides which have been isolated from natural sources and whose sulfoxide configurations have been determined are of the *S* configuration (Lucas and Levenbook, 1966; Barnsley, 1968). The single exception seems to be the class of isothiocyanate sulfoxides, $CH_3S(\rightarrow O)(CH_2)_nNCS$ (n = 3–6; 8–10) and sulforaphene (see above), found in mustard oil (Cheung *et al.*, 1965).

The various γ-glutamyl peptides including **1** disappear from the bulbs of sprouting onion and garlic and these may therefore function as nitrogen reserves (Virtanen and Matikkala, 1960b; Virtanen, 1962, 1965). No other role has apparently been proposed for these unusual peptides (see also Fowden, 1964). The occurrence of **1** in a higher plants unrelated to the *Allium* genus is surprising as is the finding that the peptide concentration is *greater* in mature plants than in young plants. The function of **1** in sandal is thus even more obscure.

Bulbs and seeds of onion and garlic lack either a γ-glutamyl peptidase or transferase capable of cleaving the γ-glu-

[6] Allyl-substituted sulfoxides were observed to depart from the rule in isooctane; in water, however, their behavior was normal.

tamyl bond in peptides of either *S*-propenyl- or *S*-allylcysteine sulfoxide, although a γ-glutamyl peptidase is present in *sprouting* bulbs and in germinating seeds of chives (Matikkala and Virtanen, 1965). Subsequent action of alliinase on the cleaved peptide results in C-S bond cleavage and deamination, the products being pyruvate, ammonia, and either the lacrimatory factor (**3**) (onion) or allicin (garlic). In the case of sandal, no lacrimation was noticeable when handling leaves, homogenates, or aqueous extracts so it is possible that the enzymes for converting **1** → **2** → **3** are absent in the leaves.

Peptide **1** in sandal probably originates from the reaction of glutathione with methacrylic acid or some equivalent (Suzuki *et al.*, 1962) followed by cleavage of glycine to **4**, an oxidative decarboxylation to **5**, and, lastly, oxidation to the sulfoxide. Alternatively, cysteine might trap methacrylic acid, receiving at some point in the pathway a glutamyl group from glutathione in a nonspecific transpeptidation (*cf.* Fowden, 1964). These pathways were postulated (Virtanen, 1962, 1965) for **1** and related compounds in onion and received support from the isolation of *S*-(2-carboxypropyl)cysteine -γ-glutamylcysteine (**4**) and -glutathione from onion (Matikkala and Virtanen, 1967, 1970; Virtanen and Matikkala, 1960a,b, 1961a,b).

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A Potent Interferon Inducer Derived from Poly(7-deazainosinic acid)†

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ABSTRACT: To determine whether or not purine N-7 of poly(I) plays a significant role in the induction of interferon by poly(I) · poly(C), poly(7-deazainosinic acid)[poly(c^7I)] was prepared by the *Micrococcus luteus* polynucleotide phosphorylase catalyzed polymerization of 7-deazainosine 5′-diphosphate, synthesized from 7-deazainosine (7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ol). Poly(c^7I) was, like poly(I), degraded to the nucleoside or nucleotide level by T_1 ribonuclease, bovine spleen phosphodiesterase, snake venom phosphodiesterase, micrococcal nuclease, and 0.3 N KOH but was totally resistant to degradation by pancreatic ribonuclease A. Unlike poly(I), poly(c^7I) showed little temperature-dependent hyperchromicity in 1.0 M NaCl with an indication of structure only below room temperature. Mixing curves as a function of wavelength, isosbestic points, and sedimentation velocity studies demonstrated that poly(c^7I) forms only 1:1 stoichiometric complexes with both poly(C) and poly(br^5C). Poly(C) · poly(c^7I) had a T_m of 49° (0.2 M NaCl, pH 7) and poly(br^5C) · poly(c^7I) had a T_m of 86° (0.2 M NaCl, pH 7). For comparison purposes, the previously reported poly(br^5C) · poly(I) complex was also prepared. These complexes were evaluated for antiviral activity and interferon inducing ability. With primary rabbit kidney cells, the following sequence (in order of decreasing activity) was established when direct inhibition of vesicular stomatitis virus cytopathogenic effect and interferon production in normal, interferon primed and superinduced (cycloheximide and actinomycin D) rabbit kidney cells were measured: poly(c^7I) · poly(br^5C) > poly(I) · poly(br^5C) > poly(I) · poly(C) > poly(c^7I) · poly(C). On the other hand, if the components of the complexes were administered sequentially followed by measurement of inhibition of virus cytopathogenic effect, the sequence (in order of decreasing activity) changed to: poly(I) · poly(C) > poly(c^7I) · poly(br^5C) > poly(I) · poly(br^5C) > poly(c^7I) · poly(C), if either poly(I) or poly(c^7I) were added to the cells first. If either poly(br^5C) or poly(C) were administered first, the order of decreasing activity was: poly(I) · poly(C) > poly(I) · poly(br^5C) > poly(c^7I) · poly(br^5C) > poly(c^7I) · poly(C).

The synthesis and biological evaluation of a number of modified polynucleotides have rendered possible the definition of several structural features required for an effective interferon inducer (Vilcek *et al.,* 1968; Colby and Chamberlin, 1969; De Clercq *et al.,* 1969, 1970, 1972a, 1974b; Steward *et al.,* 1972; Black *et al.,* 1972; Torrence *et al.,* 1973a,b; De Clercq and Janik, 1973). While a number of modifications have involved the pyrimidine base of poly(I) · poly(C) (Colby and Chamberlin, 1969; De Clercq *et al.,* 1972a; Reuss, K. and Scheit, K. H., personal communication, 1973; Folayan and Hutchinson, 1974; Johnston *et al.,* 1974) or poly(A) · poly(U) (Torrence *et al.,* 1973a; De Clercq *et al.,* 1974b), with one exception (De Clercq *et al.,* 1974), no nuclear modification involving the purine base of either complex has been reported. Since there is evidence to indicate that the purine member of poly(I) · poly(C) may be of greater importance in the induction process (De Clercq and De Somer, 1972; Carter *et al.,* 1972; Mohr *et al.,* 1972; De Clercq *et al.,* 1973), we have initiated an investigation into the effects of such nuclear modifications on the ability of polynucleotides to function as interferon inducers. In this paper, we report the synthesis, physical properties, and biological activity of one such modified polynucleotide in which N-7 of the hypoxanthine base of poly(I)[1]

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[1] Abbreviations for synthetic polynucleotides conform to the recommendations of the IUPAC-IUB Commission ((1970), *Biochemistry 9,* 4025). Thus poly(I) represents poly(inosinic acid), poly(I) · poly(C) represents the two-stranded complex with poly(C), poly(c^7I) is poly(7-deazainosinic acid), poly(c^7A) is poly(7-deazaadenylic acid), etc. Other abbreviations are as follows: T_m, temperature at the midpoint of the absorbancy change; CPE, cytopathogenic effect; PRK cells, primary rabbit kidney cells; VSV, vesicular stomatitis virus; MEM, minimal Eagle's medium; MIC, minimum inhibitory concentration; PFU, plaque forming units.