Separation and Properties of Spirographis and Isospirographis Porphyrin Dimethyl Esters†

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ABSTRACT: The oxidation of protoporphyrin IX dimethyl ester with potassium permanganate produces a mixture of spirographis (2-formyl-4-vinyl-), isospirographis (2-vinyl-4-formyl-), and 2,4-diformyldeuteroporphyrin IX dimethyl esters. These porphyrin mixtures were successfully separated by thin-layer chromatography on silica gel. The absorption spectra of these porphyrin esters showed increasing red shifts as the number of formyl groups increased. The light absorption, fluorescence, and infrared spectra of the two isomers of monoformyl-monovinyl porphyrin dimethyl esters were identical and exhibited spectra intermediate between those of proto- and 2,4-diformyldeuteroporphyrin IX dimethyl esters. The nuclear magnetic resonance (nmr) spectra, melting points, and solubilities of these isomers, however, showed considerable differences. Each isomer had only one of the two formyl proton resonances observed in 2,4-diformyldeuteroporphyrin IX dimethyl ester. The melting points of spirographis, isospirographis, and 2,4-diformyldeuteroporphyrin IX dimethyl esters were 276–278, 225, and 284–286°, respectively. The solubility of isospirographis porphyrin dimethyl ester was several fold higher than that of spirographis porphyrin dimethyl ester in various organic solvents. The effects of these solubility differences on the rate of production of the two porphyrin esters were discussed. Comparison of the rates of decomposition of various porphyrin esters on irradiation with visible light showed that protoporphyrin dimethyl ester was extremely light sensitive and decomposed to green compounds, while 2,4-diformyldeuteroporphyrin dimethyl ester was light resistant. The light sensitivities of the two isomers of monoformyl-monovinyl porphyrins were similar and were intermediate between those of proto- and 2,4-diformyldeuteroporphyrins.

Formylporphyrins are known to occur naturally as their iron complexes in the prosthetic group of cytochrome oxidase and chlorocruorin (Falk, 1964). The absorption spectra of these hemoproteins are shifted to longer wavelengths due to electron withdrawal by the formyl side chain. Formylporphyrins have also been prepared chemically by the formylation of deuterohemin (Fischer and Wecker, 1941), by the oxidation of protoporphyrin IX dimethyl ester with osmium tetroxide, hydrogen peroxide (Fischer and Deilmann, 1944; Lemberg and Falk, 1951), periodate (Sparatore and Mauzerall, 1960), or with potassium permanganate (Lemberg and Parker, 1952; Caughey *et al.*, 1966a), and by cyclization of pyrrole rings (Jackson *et al.*, 1967; Bamfield *et al.*, 1968; Clezy and Diakiw, 1973).

Attempt to separate the two isomers of monoformyl-monovinyl porphyrins were first made by Fischer and Deilmann (1944) on the products of oxidation of protoporphyrin IX dimethyl ester with osmium tetroxide and hydrogen peroxide. Lemberg and Parker (1952) also attempted a similar separation on the oxidation products with potassium permanganate, but no definite evidence was obtained for the presence of isospirographis porphyrin dimethyl ester. Caughey *et al.* (1966a) reported that two isomers of monoformyl-monovinyl porphyrins could be distinguished by the nuclear magnetic resonance (nmr) spectra, although they did not separate such isomers. Inhoffen *et al.* (1966, 1969) prepared two isomers of photoprotoporphyrins by the photooxidation of protoporphyrin IX dimethyl ester and transformed them into spirographis and isospirographis porphyrins.

Recently, we have succeeded in the direct separation of a mixture of the two isomers of monoformyl-monovinyl porphyrin esters and 2,4-diformylporphyrin ester by thin-layer chromatography (Asakura and Sono, 1973). Using this technique we proved that the oxidation products of protoporphyrin IX dimethyl ester with potassium permanganate contain the two isomers of monoformyl-monovinyl porphyrins, and we could isolate these isomers as well as 2,4-diformylporphyrin in the pure form. The present manuscript deals with the separation, identification, and properties of spirographis, isospirographis, and 2,4-diformyldeuteroporphyrin IX dimethyl esters.

The effect of these modification on human hemoglobin will be shown elsewhere (Asakura and Sono, 1974).

Experimental Section

Protohemin chloride was purchased from Sigma and used without further purification. Other chemicals of reagent grade were obtained from Fisher Scientific Co. For infrared (ir) spectra measurements chloroform was distilled after treatment with anhydrous sodium bicarbonate and concentrated H_2SO_4. Silica gel chromatography was carried out with silica gel 60 from EM Laboratories, Inc. (70–230 mesh). Precoated thin-layer plates were purchased from quantum industries, Fairfield, N. J. (silica gel, hard Q-gel).

Measurement of Nmr, Ir, and Fluorescence Spectra. The nmr spectra of various porphyrin derivatives were measured in deuteriochloroform ($CDCl_3$) with a Varian 220-MHz nmr spectrometer equipped with a Varian-4357 Fourier transform accessary at room temperature using tetramethylsilane as an internal standard. The concentrated porphy-

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‡ T. A. is a recipient of a Career Development Award (5-K04-GM-47463) from the National Institutes of Health.

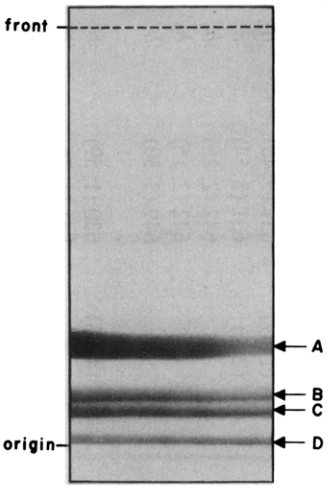


FIGURE 1: Separation of porphyrin dimethyl esters on a silica gel tlc plate. The four bands from the top to the bottom are: (A) proto-, (B) spirographis, (C) isospirographis, and (D) 2,4-diformyldeuteroporphyrin IX dimethyl esters. Chloroform was used for development.

rin solutions were prepared by the technique used for solubility measurement (see later). In order to remove undissolved porphyrin, the mixture was filtered through a cotton filter immediately before measurements. The concentration of porphyrin esters used for measurements are shown in Figure 4 legend. Ir and fluorescence spectra were measured with a Perkin-Elmer 521 ir spectrometer and Hitachi-Perkin-Elmer fluorescence spectrophotometer (MPF-2A), respectively. Melting points were determined with a Griffin and Tatlock electric micro-melting point apparatus and were not corrected. The elementary analyses were performed at Midwest Microlab. Ltd., Indianapolis, Ind.

Measurements of Time Course for Oxidation Reaction. Protoporphyrin (200 mg) was reacted with 400 mg of potassium permanganate over the time of 60 min as described below. Aliquots (10 ml) were withdrawn from the reaction mixture at an interval of 5 or 10 min, and mixed with 50 ml of chloroform. The chloroform layer was washed three times with distilled water. The first washing was reextracted with chloroform and the chloroform solutions were pooled. After treatment with anhydrous sodium sulfate, the solution was concentrated to dryness with rotatory evaporator at a temperature below 40°. The dried materials were

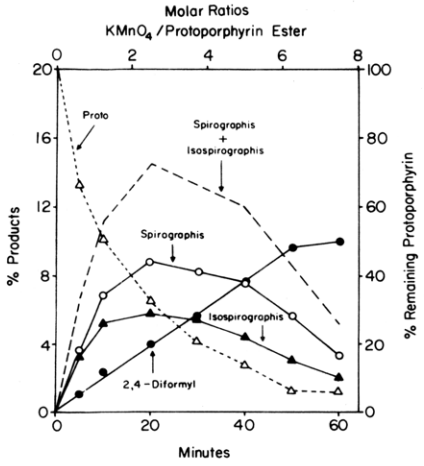


FIGURE 2: Time course of formation of various porphyrins by the oxidation of protoporphyrin IX dimethyl ester with potassium permanganate. The experimental conditions are described in the text.

TABLE I: Effects of Concentrations of Protoporphyrin IX Dimethyl Ester and Potassium Permanganate on the Yields of Formyl Porphyrin Esters.[a]

Protoporphyrin IX Dimethyl Ester (mg)	Potassium Permanganate ($KMnO_4$) (mg)	Molar Ratios ($KMnO_4$/Proto ester)	Yields (%)	
			Monoformyl	Diformyl
50	50	3.7	9.4	2.0
50	75	5.6	12.8	4.0
100	100	3.7	16.1	4.5
100	150	5.6	11.5	7.0
200	200	3.7	14.3	6.1
200	300	5.6	8.9	7.7
200	400	7.5	6.0	9.4
300	300	3.7	14.7	5.6
300	450	5.6	8.2	4.8
500	750	5.6	7.8	5.4

[a] Reactions were carried out in 800 ml of acetone as described in the text.

dissolved in 1.0 ml of chloroform, from which 100 μl were subjected to thin-layer chromatography in a narrow band on 20 × 20 cm precoated thin-layer plate (quantum industries, silica gel PQ-5, 0.5 mm thickness). The length of the band was between 2–3 cm so that about 5–8 samples can be applied on one plate. The development was made with chloroform as solvent. The four porphyrin bands on the plate from top to bottom correspond to proto-, spirographis, isospirographis, and 2,4-diformyldeuteroporphyrin IX dimethyl esters, respectively (*cf.* Figure 1). Each band was scraped and extracted with 2 ml of chloroform. After filtration through a cotton ball placed in a Pasteur pipet, the concentration of each porphyrin ester was measured spectrophotometrically.

Measurement of Solubility. The solubilities of porphyrin derivatives were determined by mixing an excess amount of porphyrin dimethyl ester into various solvents. After incubation for 2–3 hr in a sealed test tube at 20°, the mixture was filtered through a cotton filter, diluted to an appropriate concentration, and the spectrum was observed. These procedures were carried out with different excess amount of porphyrin until steady maximum values were obtained.

Preparation of Porphyrins. Protoporphyrin IX dimethyl ester was prepared from protohemin according to the meth-

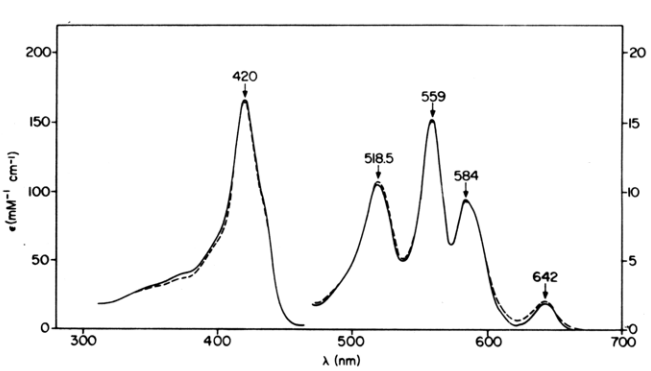


FIGURE 3: The light absorption spectra of spirographis (—) and isospirographis (- - - - - - -) porphyrin dimethyl esters in chloroform.

TABLE II: Absorption Spectra of Porphyrin Esters in Various Solvents.[a]

Solvent	Porphyrin Ester		λ (nm) (ϵ_{mM}) Soret	IV	III	II	Ia	I	Ref
Chloroform	Proto-		407 (180)	505 (14.3)	541 (12.2)	575 (7.35)	603 (1.56)	630 (5.50)	*b*
			407 (171)	505 (14.15)	541 (11.6)	575 (7.44)	603 (2.02)	630 (5.38)	*c*
			407.5 (161)	506 (13.8)	541 (11.1)	576 (6.5)		630 (5.0)	*d*
	Spirographis		420 (163)	518.5 (10.6)	559 (15.0)	584 (9.48)		642 (2.00)	*b*
			421 (161)	520 (10.6)	560 (14.8)	585 (9.33)		644.5 (2.29)	*e*
			418 (153)	515 (10.8)	555 (12.8)	584 (7.7)		642 (2.6)	*f*
	Isospirographis		420 (163)	518.5 (10.8)	559 (15.1)	584 (9.45)		642 (2.03)	*b*
			418 (183)	516 (12.5)	555 (14.6)	583 (7.7)		642 (2.3)	*f*
	2,4-Diformyl-	380 (49.2)	437 (151)	525 (14.3)	562 (8.52)	595 (7.02)		650 (3.88)	*b*
			435 (137.5)	526 (12.6)	562.5 (7.70)	595 (6.48)		651 (3.48)	*e*
			437 (134)	526 (12.5)	562 (7.3)	594 (6.0)		649 (3.3)	*d*
Ether	Proto-		403 (177)	503 (15.9)	536 (12.8)	577 (7.15)	605 (1.62)	630 (7.22)	*b*
			404 (158)	503 (14.8)	536 (11.86)	576 (6.63)	605 (1.54)	633 (6.57)	*c*
	Spirographis		414 (166)	514 (12.7)	553 (16.4)	582 (9.00)		642 (2.44)	*b*
	Isospirographis		414 (165)	514 (12.9)	553 (16.0)	582 (8.75)		642 (2.46)	*b*
	2,4-Diformyl-	376[g]	429	518	556	590		645	*b*
Dioxane	Proto-		405 (173)	504 (15.6)	538 (12.4)	576 (7.09)	603 (1.22)	631 (6.06)	*b*
			406 (164)	504 (14.7)	538 (11.59)	575 (6.86)	603 (1.41)	631 (5.60)	*c*
	Spirographis		416 (161)	515 (11.9)	554 (14.3)	582 (8.64)		640 (2.34)	*b*
				514 (18.23)	553 (21.98)	581 (14.02)		639 (4.06)	*h*
	Isospirographis		416 (166)	515 (12.3)	554 (14.1)	582 (8.82)		640 (2.27)	*b*
	2,4-Diformyl-	377 (46.2)	430 (153)	520 (13.6)	556.5 (8.12)	590 (6.42)		647 (3.42)	*b*
Pyridine	Proto-		408.5 (169)	506 (15.2)	540.5 (12.1)	576 (7.26)	604 (1.47)	631 (5.48)	*b*
			409 (163)	506 (14.8)	541 (11.87)	576 (7.48)	605 (2.0)	631 (5.54)	*c*
	Spirographis		420.5 (158)	518 (11.3)	557 (14.1)	584 (8.92)		642 (2.26)	*b*
	Isospirographis		420.5 (160)	518 (11.4)	557 (14.0)	584 (8.48)		642 (2.16)	*b*
	2,4-Diformyl-	378 (46.1)	435 (152)	524 (14.15)	560 (8.65)	593 (7.16)		649 (3.30)	*b*
Acetic acid glacial	Proto-[i]		400 (188)	504 (6.50)	536 (10.4)	562 (13.0)	606 (4.23)	630 (1.59)	*b*
	Spirographis		415.5 (147)	517.5 (8.29)	557 (12.3)	584 (9.39)		641 (1.76)	*b*
	Isospirographis		415.5 (145)	517.5 (8.40)	557 (12.3)	584 (9.49)		641 (1.80)	*b*
	2,4-Diformyl-	377 (47.7)	433 (127)	524 (12.4)	560 (7.95)	594 (6.82)		649 (3.20)	*b*

[a] The absorption spectra were measured with a Perkin-Elmer Coleman spectrophotometer at 20°. [b] Present paper. [c] Falk (1964). [d] Caughey *et al.* (1966b). [e] Lemberg and Parker (1952). [f] Inhoffen *et al.* (1969). [g] The ε values were not measured because of the extremely low solubilities. [h] Stern and Molvig (1936). [i] Spectra of the mixture of neutral and monocation forms.

TABLE III: Absorption Spectra pf Porphyrin Dications (Esters) in HCl.[d]

Porphyrins	Concn of HCl (N)	λ (nm) (ϵ_{mM}) Soret	II	I	Ref
Proto-	1	407[a]	555	598.5	*c*
	2.7	409 (263)	555.5 (14.7)	600 (5.65)	*c*
		408 (262)	554 (13.5)	598 (5.78)	*b*
	6	411 (272)	556.5 (15.9)	601 (6.06)	*c*
Spirographis	2.7	417 (228)	563 (11.0)	613.5 (6.70)	*c*
	6	419 (244)	565 (11.8)	615 (7.29)	*c*
Isospirographis	2.7	418 (218)	562 (11.9)	612 (6.56)	*c*
	6	419 (235)	564 (12.8)	613.5 (7.33)	*c*
2,4-Diformyl-	2.7	425[a]	566	613	*c*
	6	428 (247)	569 (15.4)	616 (6.02)	*c*

[a] The ϵ values were not measured because of the extremely low solubilities of the porphyrin esters. [b] Falk (1964). [c] Present paper. [d] Porphyrin esters are partially hydrolyzed to the free porphyrins in 6 N HCl.

od of Grinstein (1947) with slight modifications. We avoided use pyridine or aqueous alkaline solution to dissolve hemin since they prolonged the reaction time. Protoporphyrin dimethyl ester was purified by column chromatography on silica gel with chloroform. The crystalline product showed no detectable impurity as judged by the measurements of absorption, ir, nmr, and fluorescence spectra, melting point, and elementary analysis.

Preparation of Spirographis (2-Formyl-4-vinyl-), Isospirographis (2-Vinyl-4-formyl-), and 2,4-Diformyldeuteroporphyrin IX Dimethyl Esters. STEP 1. OXIDATION OF PROTOPORPHYRIN IX DIMETHYL ESTER WITH POTASSIUM PERMANGANATE. Protoporphyrin IX dimethyl ester (200 mg, 0.34 mol) was dissolved in 800 ml of acetone in a 2-l. tri-neck round flask on a boiling water bath. An acetone solution containing 200 mg (1.27 mol) of finely powdered potassium permanganate was added from a separatory funnel dropwise into the boiling acetone solution at a constant rate in the course of 45 min with stirring. The reaction was monitored by measuring the absorption spectra in chloroform. The optical absorption bands due to protoporphyrin (538, 630 nm) gradually decreased and those of formyl porphyrins appeared at around 555 and 640 nm. At the end of the reaction the absorption ratio (A_{555}/A_{538}) was approximately 0.8. After the reaction, the acetone solution was filtered through a sintered glass funnel by suction in order to remove manganese dioxide and other insoluble materials. The filtrate was mixed with *ca.* 400 ml of chloroform and washed with distilled water until the aqueous layer was colorless. The first aqueous layer was reextracted with chloroform and washed with water. The chloroform extracts were combined, dried over anhydrous sodium sulfate, and evaporated to dryness. This oxidation step was repeated ten times to accumulate enough material to proceed to the next step.

STEP 2. COLUMN CHROMATOGRAPHY ON SILICA GEL. The concentrated chloroform solution containing various porphyrin esters was applied on a silica gel column (4 cm × 25 cm) and eluted with chloroform. Protoporphyrin was eluted in the first few fractions, followed by monoformyl- and diformylporphyrin esters. Each fraction was checked by measuring the absorption spectrum in chloroform and also by thin-layer chromatography. The first few fractions containing only protoporphyrin dimethyl ester were combined, dried, and used again as starting material. All fractions containing monoformyl-monovinyl porphyrins were combined and further purified by thin-layer chromatography as described below. The last fractions containing mainly diformylporphyrin were also combined and subjected to the following step.

STEP 3. CRYSTALLIZATION OF 2,4-DIFORMYLDEUTEROPORPHYRIN IX DIMETHYL ESTER. The last fractions of the eluate containing mainly diformylporphyrin were combined, concentrated, filtered, and crystallized by the addition of an equal volume of hot methanol. The crystals were collected by centrifugation and dried *in vacuo*.

The supernatant solution was combined with the second portion of the eluate and further purified by thin-layer chromatography as shown below. The diformylporphyrin dimethyl ester was recrystallized twice by the chloroform–methanol procedure: yield, 80 mg (4%), mp 284–286°. The melting points reported are 303–305° (Fischer and Deilman, 1944), 280° (Lemberg and Falk, 1951), 301–303° (Sparatore and Mauzerall, 1960), and 277–279° (Caughey *et al.*, 1966b). *Anal.* Calcd for $C_{34}H_{34}O_6N_4$: C, 68.67; H, 5.76; N, 9.42. Found: C, 68.48; H, 5.90; N, 9.70. Ir in $CHCl_3$, 1730 and 1665 cm^{-1} for $COOCH_3$ and CHO, respectively. Fluorescence in $CHCl_3$, 647 nm (excitation at 400 nm).

STEP 4. THIN-LAYER CHROMATOGRAPHY. The fractions containing the two isomers of monoformyl-monovinyl porphyrin esters were combined, concentrated to *ca.* 20 ml, and applied in a narrow band on preparative precoated thin-layer plate (quantum industries, hard gel Q-5, 1 mm thick, 20 × 20 cm) with a sample streaking pipet (10–15 mg of porphyrin/plate). The tlc plates were developed with chloroform in the dark at room temperature. After development for about 3 hr, the plates were dried by standing in the dark for about 1 hr. A typical chromatogram is shown in Figure 1. The four main bands from top to bottom were identified as proto-, spigrographis, isospirographis, and 2,4-diformyldeuteroporphyrin IX dimethyl esters. The R_F values for spirographis porphyrin ester varied from 0.12 to 0.2 in chloroform. Addition of a trace of methanol (0.4–1.0%) increased the R_F values of all porphyrin esters, which was especially useful for rechromatographic separation of these isomers. Each band of the porphyrin esters was carefully scraped from the plates, ground, and extracted several

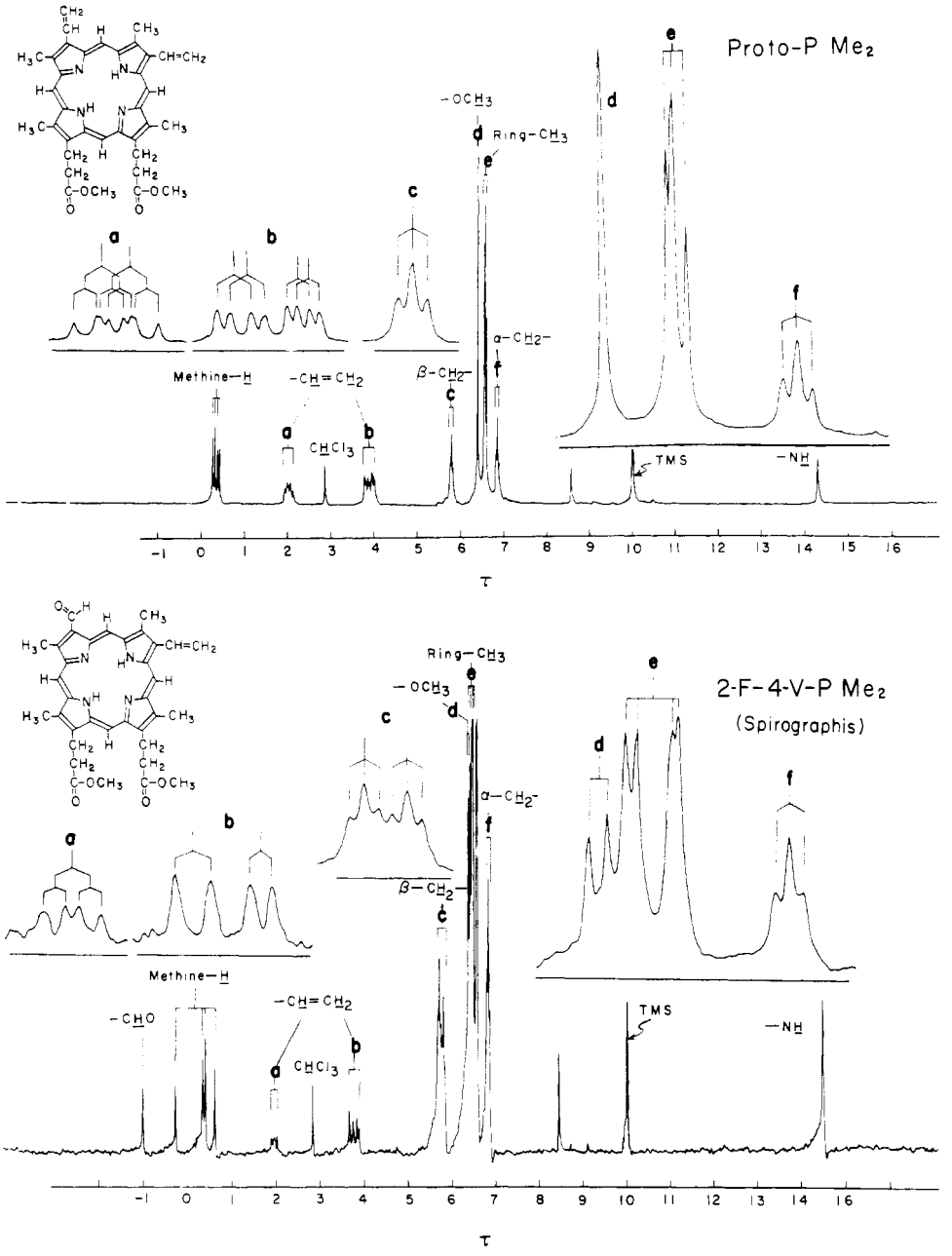


FIGURE 4: Nmr spectra of porphyrin esters. The spectra were measured with a Varian 220-MHz nmr spectrometer in $CDCl_3$ at 20°. Tetramethylsilane was used as an internal standard (τ = 10). The concentrations of the porphyrin esters were approximately 38, 18, 25, and 4 mM for proto-

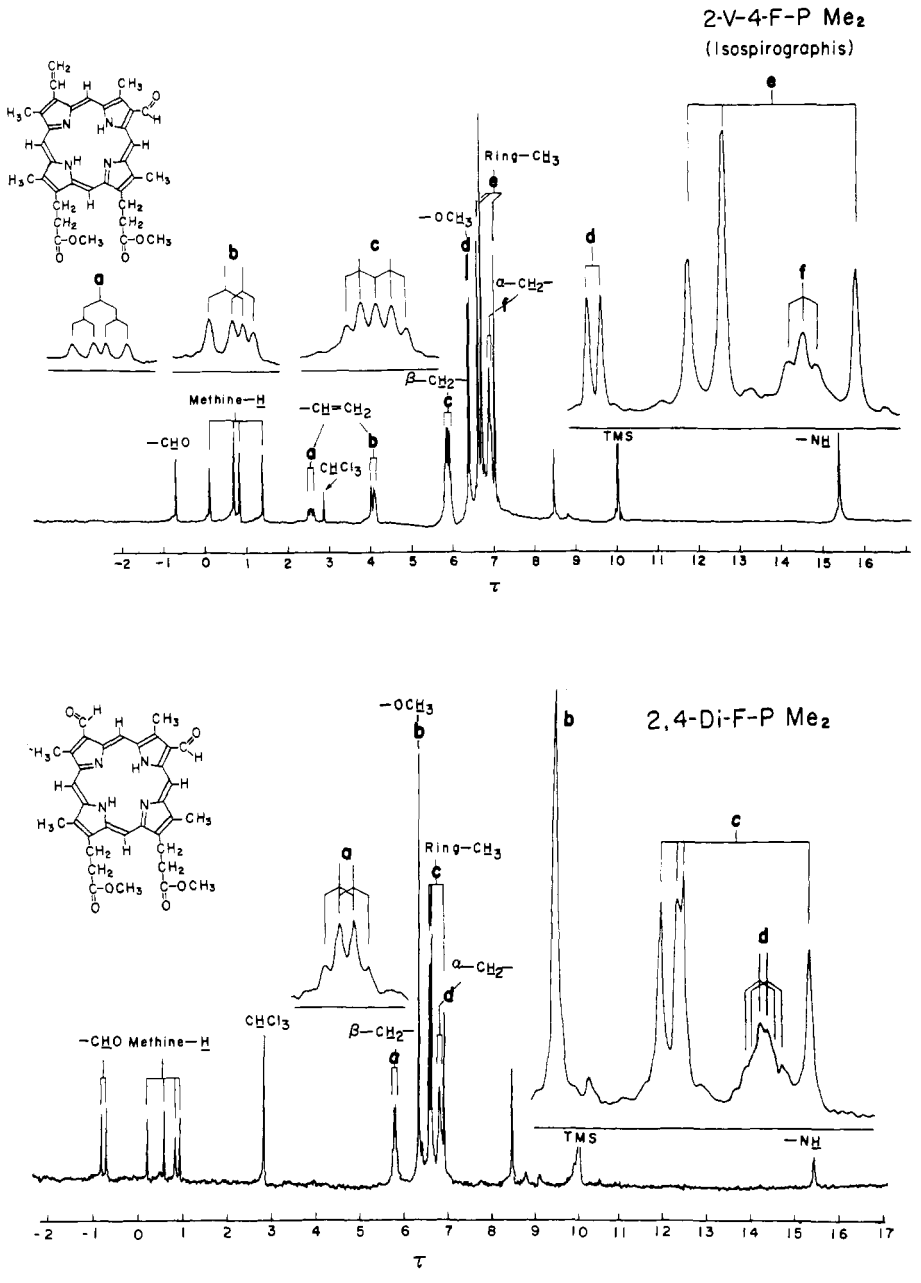
times with chloroform by filtration through the sintered glass funnel. Each fraction was concentrated to small volume (15–20 ml) and filtered again. Spirographis porphyrin ester was crystallized by adding an equal volume of hot methanol to the chloroform extract at the boiling point of chloroform. Rechromatography was necessary for the purification of isospirographis porphyrin ester, because slight contamination by spirographis porphyrin ester could not be removed by recrystallization. The solubility of spirographis porphyrin ester is lower than that of isospirographis porphyrin ester. After this second thin-layer chromatography isospirographis porphyrin ester was crystallized in a chloroform–methanol system.

Spirographis porphyrin dimethyl ester: yield, 90 mg (4.5%); mp 274–277°, 276–278° after recrystallization from chloroform–methanol. *Anal.* Calcd for $C_{35}H_{36}O_5N_4$: C, 70.93; H, 6.12; N, 9.45. Found: C, 71.20; H, 6.30; N, 9.50. Ir in $CHCl_3$, 1730 and 1660 cm^{-1} for $COOCH_3$ and CHO, respectively. Fluorescence in $CHCl_3$, 640 nm (excitation at 400 nm).

Isospirographis porphyrin dimethyl ester: yield, 76 mg (3.8%); mp 225°, not changed after recrystallization from chloroform–methanol. *Anal.* Calcd for $C_{35}H_{36}O_5N_4$: C, 70.93; H, 6.12; N, 9.45. Found: C, 70.65; H, 6.26; N, 9.56. Ir and fluorescence spectra were identical with those of spirographis porphyrin dimethyl ester.

Results and Discussion

Time Course of Oxidation Reaction of Protoporphyrin IX Dimethyl Ester. The oxidation of protoporphyrin IX dimethyl ester with potassium permanganate to diformylporphyrin dimethyl ester is expected to take place *via* two isomers of monoformyl porphyrins. In order to examine the relative importance of these two pathways, the time courses of the formation of the monoformyl porphyrins were compared to the decrease of protoporphyrin dimethyl ester, and



spirographis, isospirographis, and 2,4-diformyldeuteroporphyrin IX dimethyl esters, respectively.

the results are shown in Figure 2. The formation of the two isomers of monoformyl porphyrins reached a maximum in 20 min and then started to decrease. Although the rates of the production of the two isomers were equal at the beginning of the reaction, the level of spirographis porphyrin became higher after 5 min. This may be attributed to the difference in the solubilities of these two isomers. As shown later in this manuscript, the solubilities of isospirographis porphyrin ester in acetone is *ca.* five times higher than that of spirographis porphyrin ester. Since the volume of acetone used is not sufficient to dissolve all of monoformyl-monovinyl porphyrin esters produced, the more soluble isospirographis porphyrin ester may be oxidized at a faster rate than is spirographis porphyrin ester. This assumption was confirmed by increasing the relative volume of the reaction mixture; when we decreased the amount of both protoporphyrin and potassium permanganate from 500 to 50 mg, the molar ratio of the two isomers (spirographis/isospirographis porphyrin ester) was decreased from 2.0 to 1.2 after 45 min reaction time. These results suggest that there is no significant difference in the rate of oxidation of two or four vinyl groups of protoporphyrin although it has been suggested before that the vinyl group at 2 position is more reactive than that at 4 position by Barrett and Clezy (1959). Caughey *et al.* (1966a) also reported that the relative amounts of the two isomers were nearly equal by measuring the nmr spectra of the mixture.

Since the formyl groups can be further oxidized to carboxyl groups (Caughey *et al.*, 1966a), if we increase the amount of potassium permanganate relative to protoporphyrin, the yields of formyl porphyrins decrease. The effects of different protoporphyrin concentrations on the formation of mono- and diformylporphyrin dimethyl ester are compared in Table I. The yields of total formylporphyrins decreased either when the amounts of protoporphyrin dimethyl ester and potassium permanganate were increased

TABLE IV: Nmr Spectra (τ Values) of Porphyrins in Deuteriochloroform $(CDCl)_3$.[a,b]

Protons		Protoporphyrin IX Dimethyl Ester	Spirographis (2F-4V) Porphyrin Dimethyl Ester	Isospirographis (2V-4F) Porphyrin Dimethyl Ester	2,4-Diformyl-deutero-porphyrin IX Dimethyl Ester
Formyl	CHO	—	−1.04	−0.72	−0.88
					−0.77
Methine ($\alpha,\beta,\gamma,\delta$)	H	0.24	−0.30	0.10	0.14
		0.29	0.31	0.68	0.51
		0.35	0.36	0.82	0.77
		0.40	0.59	1.37	0.87
Vinyl	$CH{=}CH_2$ (A)	1.96 (q) (A)	1.94 (q) (A)	2.56 (q) (A)	
		2.02 (q) (A′)			
Vinyl	$CH{=}CH_2$ (B,C)	3.78 (d) (B)	3.68 (d) (B)	4.07 (d) (B)	
		3.81 (d) (B′)	3.84 (d) (C)	4.11 (d) (C)	
		3.94 (d) (C)			
		3.96 (d) (C′)			
β-Methylene	$CH_2CH_2C(O)OCH_3$	5.76 (t, 4 H)	5.68 (t, 2 H)	5.85 (t, 2 H)	5.74 (t, 2 H)
			5.77 (t, 2 H)	5.92 (t, 2 H)	5.78 (t, 2 H)
Prop-	OCH_3	6.39 (s, 6 H)	6.35 (s, 3 H)	6.36 (s, 3 H)	6.30 (s, 6 H)
			6.38 (s, 3 H)	6.40 (s, 3 H)	
Ring(1,3,5,8)	CH_3	6.53 (s, 3 H)	6.42 (s, 3 H)	6.59 (s, 3 H)	6.53 (s, 3 H)
		6.56 (s, 6 H)	6.45 (s, 3 H)	6.66 (s, 6 H)	6.56 (s, 3 H)
		6.58 (s, 3 H)	6.53 (s, 3 H)	7.00 (2, 3 H)	6.58 (s, 3 H)
			6.55 (s, 3 H)		6.87 (s, 3 H)
α-Methylene	$CH_2CH_2C(O)OCH_3$	6.83 (t, 4 H)	6.80 (t, 4 H)	6.95 (t, 4 H)	6.76 (t, 2 H)
					6.78 (t, 2 H)
	NH	14.27	14.47	15.40	15.29

[a] The τ values were obtained from the nmr spectra shown in Figure 5. [b] Spin coupling constant: J_{AB} $(= J_{A'B'})$ = 17.5 Hz, J_{AC} $(= J_{A'C'})$ = 11.5 Hz. s, singlet; d, doublet; t, triplet; q, quartet; all other peaks are singlet.

to more than 500 mg or decreased to less than 50 mg in the starting acetone volume (800 ml). The highest yield was obtained when 200–300 mg of protoporphyrin dimethyl ester was reacted with approximately the same weight of potassium permanganate.

Absorption Spectra of Formylporphyrins. Spirographis and isospirographis porphyrin dimethyl esters in chloroform show the rhodo-type spectra (III > IV > II > I) (Caughey *et al.*, 1966b), while proto- and 2,4-diformylporphyrin dimethyl esters exhibit the etio-type spectra (IV > III > II > I).

There was no significant difference between the spectra of two isomers indicating that the effects of modification on the electronic structure of porphyrins are equal at the 2 and 4 positions of the porphyrin ring (Figure 3). The optical properties of the two isomers of monoformyl-monovinyl porphyrin esters in various organic solvents were also identical and those are summarized in Table II, in comparison with those of proto- and 2,4-diformylporphyrin dimethyl esters. The absorption spectra of the dication forms of the four porphyrins in HCl are shown in Table III.

Nmr Spectra. The nmr spectra at 220 MHz of the four porphyrin dimethyl esters in deuteriochloroform are shown in Figure 4. The τ values for spectra are summarized in Table IV. The chemical shifts of formyl, vinyl, and methine protons were dependent on the porphyrin concentration as reported by York and Caughey (1963) and Caughey *et al.* (1966a). In the nmr spectrum of protoporphyrin IX dimethyl ester, the two groups of peaks in the region τ = 2.0 and 4.0 are attributed to the vinyl groups. The relative intensities of these peaks are decreased to half of the original intensity upon oxidation of one of the two vinyl groups to formyl groups. These peaks are completely missing in the spectrum of 2,4-diformyldeuteroporphyrin IX dimethyl ester. The new peaks appeared at an extremely low field (τ = −1.0 ~0). These peaks are undoubtedly due to the protons of the formyl groups at the 2 and 4 positions of the porphyrin ring. It is of great interest to note that the two isomers of monoformyl-monovinyl porphyrin esters have only one of these two formyl peaks indicating that the protons of the formyl groups at the 2 and 4 positions have different τ values. Since the mixture of them clearly produces two peaks in this region in $CDCl_3$, the purity of monoformyl-monovinyl porphyrin can easily be examined by measuring the nmr spectrum.

Solubilities of Porphyrin Esters. Although the optical properties of the two isomers of monoformyl-monovinyl porphyrin dimethyl esters are similar to each other, we noticed a considerable difference between the solubilities of these two isomers. As shown in Table V, protoporphyrin IX dimethyl ester was the most soluble among the four porphyrins examined, followed by isospirographis, spirographis, and 2,4-diformyldeuteroporphyrin IX dimethyl esters. In various organic solvents examined, these porphyrin esters were the most soluble in chloroform and the most insoluble in methanol. The formylporphyrin esters were extremely insoluble in ether and methanol.

The ratios of solubilities of isospirographis to spirographis porphyrin dimethyl ester were 1.3, 2.3, 3.2, 5.6, 1.6, and 6.7 in chloroform, tetrahydrofuran, dimethyl sulfoxide,

TABLE V: Solubilities (mM) of Porphyrin Esters in Various Organic Solvents at 20°.[a]

Solvents	Porphyrin Esters			
	Proto-	Spirographis	Isospirographis	2,4-Diformyl-
Chloroform	38.46 (100)	18.61 (48.4)	25.25 (65.6)	3.92 (10.2)
Dimethyl sulfoxide	0.77 (100)	0.15 (20)	0.48 (63)	0.09 (12)
Tetrahydrofuran	2.06 (100)	0.27 (13)	0.61 (30)	0.038 (2)
Ether	0.082 (100)	0.0046 (5.6)	0.029 (35.2)	<0.0007 (0.9)
Acetone	0.946 (100)	0.05 (5.3)	0.28 (29.6)	0.016 (1.7)
Ethyl acetate	0.427 (100)	0.094 (22.0)	0.150 (35.1)	0.0064 (1.5)
Methanol	0.012 (100)	<0.0004 (3.3)	0.0016 (13.3)	<0.0001 (0.8)

[a] The numbers in the parentheses show relative solubilities to protoporphyrin IX dimethyl ester.

acetone, ethyl acetate, and ether, respectively, at 20°. This result indicates that if we use the classical recrystallization method for the purification of the monoformyl-monovinyl porphyrins, the more soluble isospirographis porphyrin ester tends to remain in the mother liquor and the proportion of the spiroraphis porphyrin ester in the crystals gradually increases. This may be the reason why Fischer and Deilman (1944) and Lemberg and Parker (1952) could not separate isospirographis porphyrin from the oxidation products of protoporphyrin IX dimethyl ester.

Light Sensitivities of Various Porphyrin Derivatives. During the preparation of various porphyrin derivatives, we noticed that formylporphyrins are more stable than protoporphyrin which decomposes rapidly to green compounds in the light. It is known that protoporphyrin IX dimethyl ester in benzene, pyridine, and methylene chloride is photooxidized to 1-hydroxy-2-desvinyl-2-formylethylidene protoporphyrin dimethyl ester, which is also called photoprotoporphyrin or dioxyprotoporphyrin (Fischer and Bock, 1938; Barrett, 1959; Inhoffen *et al.*, 1966, 1969).

In order to compare the rate of light decomposition, 0.05 mM porphyrin esters dissolved in chloroform and kept in sealed test tubes were exposed to a 15-W fluorescent lamp at 30 cm distance at room temperature and the spectra changes were investigated for 5 days.

In the solution containing protoporphyrin, a new peak at 670 nm appeared in a few minutes associated with the decrease in the original porphyrin absorptions. New peaks in the longer wavelengths were also observed in spirographis (687 nm) and isospirographis (683.5 nm) porphyrin solutions. The absorption spectrum of 2,4-diformylporphyrin did not change significantly after 5 days of incubation except for the appearance of a small shoulder at 665 nm. The control experiment in the dark for these four porphyrins caused no spectral change after incubation for 5 days at room temperature. Thin-layer chromatography on silica gel with chloroform as solvent of the reaction mixtures showed that after 5 days, protoporphyrin IX dimethyl ester (R_F 0.27) was completely changed to two compounds having R_F values 0 and 0.06. The upper green material was the main product and had absorption maxima at 671, 612, and 572 nm in chloroform. This compound is assumed to be photoprotoporphyrin dimethyl ester (Inhoffen *et al.*, 1969). Monoformyl-monovinyl porphyrin esters were converted partially (<50%) to compounds having greenish brown color and these did not move on thin-layer chromatograms. The absorption maxima of the greenish brown compounds produced by photooxidation of spirographis and isospirographis porphyrin dimethyl esters were 687, 631, and 556 nm (spirographis) and 683.5, 624, and 566 nm (isospirographis), in $CHCl_3$.

The rates of the production of these photooxidized products were nearly equal indicating that there is no significant difference in the photooxidation rates of these two monoformyl-monovinyl porphyrins.

Diformylporphyrin dimethyl ester produced a trace of brown compound with R_F 0.

Inhoffen *et al.* (1966) reported that the protoporphyrin IX dimethyl ester in methylene chloride is rapidly photooxidized to approximately equal amounts of photoprotoporphyrin and isophotoprotoporphyrin dimethyl esters at high yields (>70%). The accumulation of these two isomer photooxidized porphyrins must be due to stabilization of vinyl group after oxidation of one of the two vinyl groups to a strongly electron-withdrawing group (formylethylidene). Barrett and Clezy (1959) suggested that a formyl group exerts an inhibitory effect upon the rate of oxidation of a double bond conjugated to the porphyrin nucleus. The electron-withdrawing effect of the formyl groups may be the reason why the two monoformyl-monovinyl porphyrins are more light resistant than protoporphyrin.

Identification of Two Isomers of Monoformyl-monovinyl Porphyrins. The two isomers of monoformyl-monovinyl porphyrins have similar properties in absorption, fluorescence, and ir spectra and in their mobilities on most thin-layer chromatograms. On the other hand, the isomers showed differences in their nmr spectra, solubilities, melting points, and mobilities on certain thin-layer chromatograms. These differences can be used for the identification of the two isomers.

The definite identification of spirographis porphyrin is due to work done by Fischer and Seeman (1936). They determined the structure of spirographis porphyrin as 2-formyl-4-vinyldeuteroporphyrin IX. Absolute proof the isomeric structure of spirographis porphyrin has recently been furnished by its total synthesis (Jackson *et al.*, 1967; Bamfield *et al.*, 1968).

Since the melting point of one of our products (276–278°) accords with that of spirographis porphyrin dimethyl ester reported (285, 277–278, 281–283, 276–278, and 278–279° by Fischer and Seeman, 1936; Lemberg and Parker, 1952; Jackson *et al.*, 1967; Bamfield *et al.*, 1968; and Inhoffen *et al.*, 1966, respectively), this compound must be 2-formyl-4-vinyldeuteroporphyrin IX dimethyl ester. On the other hand, isospirographis porphyrin dimethyl ester has a melting point (225°) which is lower by about 50°

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

The authors thank Drs. H. Akimoto and A. Coulson for their valuable advice and discussions. We also acknowledge Dr. J. S. Leigh and Miss K. Nolen for nmr measurements, Drs. R. Hershberg and W. Englander for ir measurements, and Dr. J. Leonard for fluorescence measurements. Technical assistance by Mr. A. Bracey is also acknowledged.

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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†

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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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