Chemical Syntheses and Properties of Hydroxycinnamoyl-Coenzyme A Derivatives

J. Stöckigt and M. H. Zenk

Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität Bochum, Bochum

(Z. Naturforsch. **30 c**, 352–358 [1975]; received February 3, 1975)

Coenzyme A Thiol Esters, Cinnamic Acids

Acyl-CoA derivatives of several hydroxylated cinnamic acids have been synthesized in 30 to 50% yield *via* a. acyl phenyl thiol esters, b. acyl N-hydroxysuccinimide esters, and c. glucocinnamoyl derivatives. Properties of the intermediates have been determined. The cinnamyol-CoA thiol esters were characterized by their chromatographic behaviour and UV spectra. The molar extinction coefficients of these important intermediates in plant phenylpropane metabolism have been unequivocally determined. Recently published values [13] for the molar extinction coefficients of these derivatives are incorrect; the methodological reason for this error has been established.

Cinnamoyl-coenzyme A thiol esters have long been discussed as central intermediates in phenylpropane metabolism in plants. The biosynthesis of flavonoid compounds is assumed to proceed *via* the condensation of cinnamoyl-CoA thiol esters either with acetyl-CoA [1] or malonyl-CoA [2]. Activated cinnamic acids have also been postulated as intermediates in the formation of lignin [3], benzoic acids [4], and other phenolics of higher plants [5].

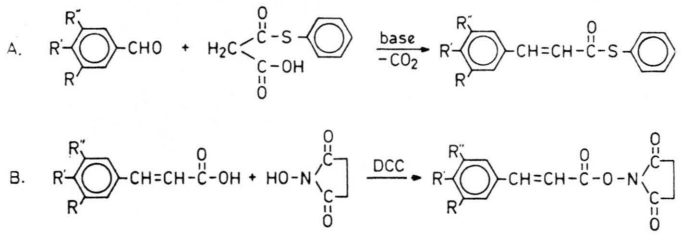
Recently proof of the participation of cinnamoyl-CoA thiol esters was found in the cell-free formation of naringenin [6], the biosynthesis of cinnamyl alcohols [7–9], and the formation of chlorogenic acid [10]. The established role of cinnamoyl-CoA thiol esters in the biosynthesis of plant phenolics made it necessary to develop methods for the chemical synthesis of these important intermediates. Previously only enzymatic methods for the preparation of cinnamoyl-CoA derivatives were available [3, 11]. These techniques give small amounts of the desired products but allow the synthesis of radioactive intermediates with high specific activity. The necessity of having larger amounts of hydroxycinnamoyl-CoA derivatives at hand, especially for the application of optical assays in reactions involving a loss of the characteristic thiol ester absorption in the long UV-region [3] and as substrates for substitution reactions, made it necessary to develop chemical methods of synthesis. Previous attempts to synthesize hydroxycinnamoyl-CoA derivatives by chemical means have failed [12], due to the reactivity of the phenolic hydroxyl group and the danger of substitution of SH-compounds to the double bond of the acryloyl side chain. Very recently Johns [13] published a method for the synthesis of cinnamoyl-CoA derivatives involving the direct synthesis of cinnamoyl phenyl thiol esters and subsequent thiol ester exchange [14, 15] onto CoA-SH. Using these derivatives, Johns [13] counterchecked some of the properties of the enzymatically synthesized cinnamoyl-CoA derivatives prepared by Gross and Zenk [3] and criticized several aspects of their work. We now present three independent chemical syntheses for the preparation of hydroxycinnamoyl-CoA derivatives with high metabolic activity and present data which prove that the analytical methods used by Johns [13] gave, in most cases, inconclusive results.

Abbreviations: CoA, Coenzyme A; DCC, Dicyclohexyl carbodiimide.

Requests for reprints should be sent to Dr. J. Stöckigt, Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität Bochum, *D-4630 Bochum*, W.-Germany.

Material and Methods

Biochemicals of the highest purity available were obtained from Boehringer, Mannheim. Radioactive cinnamic acids were synthesized by standard Knoevennagel procedures using appropriate aldehydes and [2-^{14}C]malonic acid (NEN, Boston). *p*-Coumaric, caffeic and ferulic acid had specific activities of 1.90, 1.95, and 1.85 μCi per μmol, respetively. Mass spectra were recorded on a VARIAN MAT 111. C,H,N-analyses were performed by Fa. Pascher, Bonn.



Scheme I. Synthesis of activated cinnamoyl esters.

R=R′=R″=H	cinnamoyl derivatives
R=R″=H; R′=OH	*p*-coumaroyl derivatives
R=H; R′=R″=OH	caffeoyl derivatives
R=H; R′=OH; R″=OCH_3	feruloyl derivatives
R=R″=OCH_3; R′=OH	sinapoyl derivatives

Synthesis of intermediates

a. Synthesis of cinnamoylthiophenyl esters

To the corresponding substituted benzaldehyde (1.4 mmol) was added crystalline malonyl thiophenol [16] (1.7 mmol) and the mixture dissolved in 0.4 ml of absolutely dry pyridine/piperidine (10:1). After 6 hours at room temperature, during which time the course of the reaction can be followed by observing CO_2 set free during the reaction, the solution was diluted with water and acidified with 2N hydrochloric acid. After 12 hours the mixture was exhaustively extracted with ethyl acetate. The dried extract was chromatographed on silicagel GF using $CHCl_3$/ethyl acetate/benzene/ethyl methyl ketone/light petrol (40 – 60 °C) in a ratio of 7:1:2:3:3. The thiol ester was eluted using ethyl acetate/methanol and after evaporation of the solvent the esters crystallized, except for sinapoyl phenyl thiol ester. Some properties and yields of these esters are given in Table I. UV-spectra were recorded in methanol, λ_{max} values for the maximal absorption of the thiol ester linkage are presented.

Cinnamoyl thiophenyl ester was also synthesized *via* the acid chloride and thiophenol [17]. Analysis of the cinnamoyl phenyl thiol ester m.p. 92 – 93 °C (found C: 74.73%; H: 4.9$_5$%; S: 13.35%; calcd. for $C_{15}H_{12}OS$ C: 74.97%; H: 5.03%; S: 13.34%).

b. Synthesis of N-hydroxysuccinimide esters of cinnamic acids

The cinnamic acid in question (15 mmol) was dissolved under heating in absolute ethyl acetate, N-hydroxysuccinimide (15 mmol) and, after cooling to 30 °C, dicyclohexyl carbodiimide (17 mmol) was added. After 24 hours the dicyclohexylurea was filtered off and the filtrate extracted with 1 M sodium bicarbonate. After drying the ethyl acetate phase over sodium sulfate, the solvent was evaporated and the esters purified by thinlayer chromatography (silicagel GF; solvent: $CHCl_3$/MeOH = 20:1). The esters were recrystallized from benzene. Some analytical data and yields of the pure products are given in Table II.

c. Glucocinnamic acids

β-D-Glucocinnamic acids were synthesized according to published methods [18]. Melting points agreed with published values.

Thiol ester exchange reaction and transesterification

For the synthesis of hydroxycinnamoyl-CoA from cinnamoyl thiophenyl esters or the cinnamoyl N-hydroxysuccinimide esters the following general procedure was applied. Nitrogen was bubbled through an aqueous solution (2 ml) of CoA (10 μmol) at a low rate, solid $NaHCO_3$ (100 μmol) was added and subsequently the thiophenyl (33 μmol) or N-hydroxysuccinimide ester (50 μmol) added. Acetone was added till the mixture formed a

Table I. Melting points, optical values, mass spectral characteristics, R_f values and yields for hydroxylated series of phenyl thiol esters. Chromatography on silica gel. I: in chloroform/methanol 20:1; II: in chloroform/ethyl acetate/benzene/ethyl methyl ketone/light petrol. 7:1:2:3:3.

Phenyl thiol esters	m.p. [°C]	IR [cm^{-1}]	UV λ_{max} [nm]	MS *m/e* (Intens.%)	R_f I	R_f II	Yield [%]
Cinnamoyl	92 – 93 *	1680; 1440; 1330; 1019; 750; 690	302	240(2); 131(100); 109(5); 103(33)	0.94	0.99	30
p-Coumaroyl	116 – 119	1670; 1435; 1325; 1020; 750; 686	338	256(2); 147(100); 119(19); 109(4)	0.76	0.84	20
Caffeoyl	126 – 129	1655; 1440; 1350; 1020; 737; 686	352	272(1); 163(100); 135(10); 109(8)	0.40	0.59	23
Feruloyl	72 – 75	1660; 1430; 1380; 1018; 740; 687	352	276(2); 177(100); 149(6); 109(5)	0.90	0.85	22
Sinapoyl	oil	1665; 1430; 1340; 1022; 748; 690	356	316(2); 207(100); 175(31); 109(3)	0.90	0.78	11

* Literature value [17]: 91 °C; Johns [13]: 90 – 91 °C.

Table II. Melting points, optical values, mass spectral characteristics, R_f values and yields for hydroxylated series of N-hydroxysuccinimide esters. Chromatography on silica gel. I: in chloroform/methanol 20:1; II: in chloroform/ethyl acetate/benzene/ethyl methyl ketone/light petrol. 7:1:2:3:3.

N-Hydroxy-succinimide esters	m.p. [°C]	IR [cm^{-1}]	UV λ_{max} [nm]	MS *m/e* (Intens.%)	R_f I	R_f II	Yield [%]
Cinnamoyl	180–182	1760; 1740; 1628; 1370; 1073; 644	286	245 (2); 131 (100); 103 (30)	0.89	0.67	81
p-Coumaroyl	184–188	1760; 1710; 1620; 1378; 1071; 645	332	261 (2); 147 (100); 119 (23)	0.42	0.37	36
Caffeoyl	176–179	1765; 1725; 1630; 1370; 1070; 660	342	277 (4); 163 (100); 135 (13)	0.23	0.17	15
Feruloyl	145–148	1760; 1725; 1630; 1378; 1070; 645	341	291 (9); 177 (100); 145 (74)	0.66	0.35	30
Sinapoyl	116–118	1750; 1730; 1620; 1378; 1070; 650	344	321 (15); 207 (100); 175 (46)	0.66	0.29	52

single phase. The solutions were kept at 4 °C for a total of 24 hours. The organic solvent was evaporated by a stream of nitrogen. The aqueous phase was desalted with Dowex 50 W-X8, extracted with ethyl acetate, freeze dried, and the residue purified by chromatography. Yields, estimated spectroscopically (UV), ranged from 30 to 50% for the hydroxycinnamoyl-CoA derivatives based on CoA.

Cinnamoyl-CoA derivatives via gluco-cinnamoyl-CoA's

Glucose derivatives of hydroxycinnamic acids were esterified in the presence of CoA (10 to 50 μmol) and dicyclohexyl carbodiimide by the technique used for the synthesis of feruloyl-AMP [19]. Gluco-hydroxycinnamoyl-CoA derivatives were isolated in 35 – 50% yield. These derivatives were incubated at pH 5.0 with β-glucosidase (Boehringer, Mannheim, which proved to be absolutely free of thiol esterase activity) and kept for 20 min at 30 °C. Gluco-hydroxycinnamoyl-CoA derivatives were thus transformed into hydroxycinnamoyl-CoA derivatives in essentially quantitative yield. Isolation and purification of the CoA thiol esters was done by chromatography (Table III).

Identification of hydroxycinnamoyl-CoA thiol esters

The reaction products were checked for their identity as cinnamoyl-CoA thiol esters by several procedures. Ascending chromatography was done at room temperature on Whatman No. 3 paper in the solvent systems given in Table III. The hydroxycinnamoyl-CoA thiol esters gave a characteristic yellow color upon treatment with nitroprusside reagent under alkaline conditions [20] as previously observed for this class of compounds [3]. Free phenolic OH-groups were detected by spraying with diazotized sulfanilic acid. The thiol esters yielded the corresponding hydroxamic acids upon treatment with hydroxylamine at pH 7.0. The most sensitive assay for the purified derivatives was their characteristic UV-spectrum recorded in 0.1 M potassium phosphate buffer at pH 7.0 in combination with the change in spectral characteristics after hydrolysis of the thiol ester linkage [3].

Hydroxamate test [21]

For the quantitative determination of hydroxycinnamoyl thiol esters the hydroxamate procedure by Johns [13] was followed exactly. For the purified phe-

Table III. R_f values of hydroxycinnamic acids, their CoA thiol esters and hydroxamic acids on paper. I. in *n*-butanol/glac. acetic acid/water 5:2:3; II: in *i*-butyric acid/ammonia/water 66:1:33; III: in ethanol/0.1 N sodium acetate pH 4.5 1:1; IV: in *n*-butanol/glac. acetic acid/water 20:1:4.

Acid	Parent acid I	Parent acid II	Parent acid III	CoA thiol ester I	CoA thiol ester II	CoA thiol ester III	Hydroxamic acid IV
Cinnamic	0.94	0.92	0.85	0.43	0.59	0.75	0.83
p-Coumaric	0.85	0.86	0.85	0.58	0.42	0.75	0.69
Caffeic	0.72	0.77	0.52	0.37	0.42	0.66	0.45
Ferulic	0.77	0.83	0.79	0.46	0.40	0.68	0.60
Sinapic	0.77	0.85	0.66	0.34	0.66	0.70	0.42

nyl thiol esters we used, 0.5 ml of 0.2 M hydroxylamine (pH 8.0) which corresponds to 100 μmol NH_2OH per test (Johns, pers. commun.). Cinnamoyl hydroxamate was synthesized from cinnamoyl ethyl ester, using a general method [22]. The hydroxamate was recrystallized from H_2O/MeOH. The pure compound had a m.p. of 117.5 – 119 °C. Analysis of the product gave C: 65.94%; H: 5.41%; N: 8.54% (calcd. for $C_9H_9NO_2$ C: 66.25%; H: 5.56%; N: 8.58%).

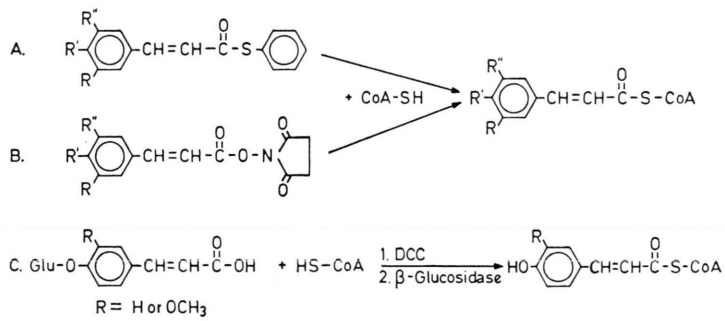
Purification of hydroxycinnamoyl-CoA derivatives

For small scale purification of hydroxycinnamoyl-CoA derivatives paper chromatography using the solvent systems given in Table III, proved very useful. Since column chromatography of these derivatives on Sephadex G-10 [11] did not work satisfactorily in our hands, larger amounts of synthetic cinnamoyl derivatives were chromatographed at 4 °C on DEAE cellulose columns (2.5 × 8 cm), using an HCOOH/HCOONa gradient [23]. The effluent of the column was monitored automatically and simultaneously at 254 nm and 339 nm. The CoA thiol ester containing fractions showed peaks at both wavelengths. The pooled fractions containing the CoA thiol ester were isolated as in l.c. [23] and kept in the dry state at – 20 °C; under these conditions deterioration of the compound was minimal. Yields of the CoA thiol ester purified by this method were about 75% of the applied compound.

Results and Discussion

Acids containing one or more free phenolic groups in the aromatic ring system and a double bond in the side chain present difficult problems in the chemical esterification with coenzyme A or with other thiols. Problems arise using either carboxyl activation reactions or coupling agents. Since attack at the free phenolic hydroxyl is likely to occur [12] and Michael addition of the thiol to the double bond of the cinnamic acid is a possibility [20]. In both cases these side reactions would interfere with formation of the desired thiol esters. To circumvent these difficulties, three independent syntheses have been developed. To avoid the use of free thiol, and therefore the possibility of an addition of SH-groups to the double bond of the cinnamic acids, malonyl thiophenol was condensed onto a properly substituted benzaldehyde in a Knoevennagel reaction according to Scheme I, A. This reaction gave a satisfactory yield of the desired thiol ester intermediate (Table I). Johns [13] stated that Michael addition of SH-groups to the double bond of cinnamic acids is not a serious problem. This observation was made independently by us during the course of this work. However, direct reaction of hydroxycinnamic acids with thiophenol in the presence of DCC according to the procedure of Johns [13] yielded after thinlayer chromatography, at least five different products in about equal amounts. One of these products was the desired thiol ester. Johns [13] did obtain these phenyl thiol esters of hydroxycinnamic acids by his method, but apparently not in crystalline form since no mention was made in his paper of the analytical properties of these intermediates. Thus, we consider that the condensation of malonyl thiophenol onto benzaldehydes, to yield cinnamoyl phenyl thiol esters is superior to the direct method [13].

Chemical protection of the free phenolic groups of cinnamic acids during the preparation of CoA thiol esters is difficult since the chemical removal of the protecting group, after the thiol ester formation had been achieved, would in most cases lead to a destruction of the labile ester linkage [12]. Protection of the free phenolic group by glucosidation was attempted by the technique previously described for the synthesis of feruloyl-AMP [19]. One of the prerequisites for a successful employment of this reaction is that the β-glucosidase be absolutely free of thiol esterase which was fortunately the case for our commercial sample.



Scheme II. Synthesis of cinnamoyl CoA thiol esters.

β-D-Gluco-*p*-coumaric acid and β-D-glucoferulic acid were coupled directly with CoA using DCC. The resulting thiol ester glucoside was isolated by chromatography and the glucose moiety subsequently split off by treatment with β-glucosidase as shown in Scheme II, C. The hydroxycinnamoyl-CoA derivatives thus obtained were identical in all respects to those synthesized by alternative chemical or enzymatic means. Thus glucose can be used as protecting group in the synthesis of very labile biochemical intermediates of phenols.

Since N-hydroxysuccinimide esters of aliphatic acids were shown to present useful activated intermediates for the formation of aliphatic acyl-CoA thiol esters [24], we attempted the direct synthesis of hydroxycinnamoyl N-hydroxysuccinimide esters using DCC without protection of the phenolic group of the cinnamic acids in question. It was hoped that the reactive hydroxysuccinimide would, under these conditions, attack the carboxyl group of the acids more readily than the phenolic group. This was indeed the case in spite of the fact that quite a number of other derivatives were also formed. The desired esters were, however, readily isolated by preparative chromatography in satisfactory yields (Table II).

Thiol ester exchange of the phenyl thiol ester, as well as transesterification of the hydroxysuccinimide esters under the same conditions as given in the material and methods section, gave the corresponding CoA thiol esters in about 40% yield. Unreacted esters were removed by ethyl acetate extraction and the CoA esters purified by either paper or column chromatography. Table III shows the R_f-values of parent acids as compared with their CoA thiol esters in three different solvent systems which had been used previously for the purification of enzymatically synthesized cinnamoyl-CoA esters [3]. The previously published R_f-values [3] agreed reasonably well with those reported here. We cannot explain why Johns [13] had difficulties in using two of the three reported chromatographic solvent systems [3], especially since the butanol:glacial acetic acid:H_2O (5:2:3) system gives extraordinary sharp spots and separation and has been used by others for the separation of a great many acyl-CoA derivatives even on thinlayer plates [25]. The main purpose of the chromatographic purification reported above is the separation of parent acids from CoA derivatives which is achieved in every case. Separation of different hydroxycinnamoyl-CoA derivatives from each other will only rarely occur *in praxi*.

Further identification of the synthetic CoA derivatives was done as described in material and methods. The "delayed" nitroprussid reaction and the formation of hydroxamates and their chromatographic properties (Table III) proved especially useful to supplement the optical characteristics of the esters. Since the UV-spectra of both caffeoyl-CoA and sinapoyl-CoA have not been published previously these curves are given in Fig. 1. These synthetic cinnamoyl-CoA thiol esters are metabolically active in two different enzyme assays [9, 10].

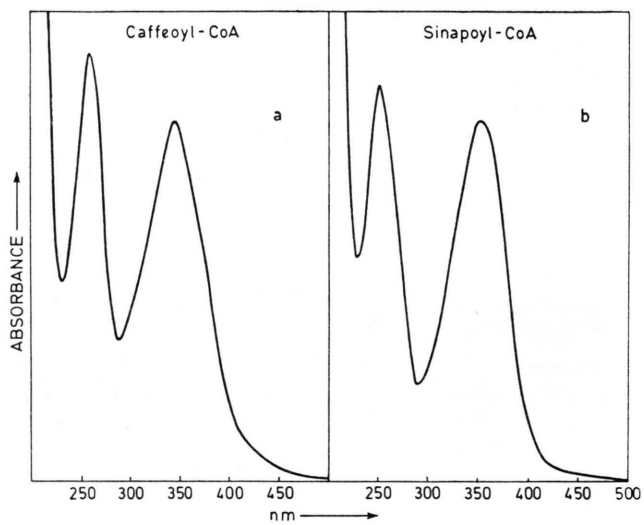


Fig. 1. UV-spectrum of caffeoyl-CoA (a) and sinapyl-CoA (b) both recorded in 0.1 M phosphate buffer pH 7.0.

The chemical preparation of hydroxycinnamoyl-CoA thiol esters by the above methods is suitable for large quantities of the CoA esters in question. For the preparation of radioactively labelled CoA esters, especially of high specific activity, the enzymatic methods [3, 11] are preferred. Of great importance is the reliability of the quantitative hydroxamate color test for cinnamoyl-CoA derivatives. The reason is twofold. First, several groups still use [26], or used this test in the past [27], for the assay of hydroxycinnamate:CoA ligase. Amounts of hydroxamates formed were calculated [11, 28] from the extinction coefficient for cinnamoyl hydroxamate as determined by Gross and Zenk [3]. Secondly, Johns [13] computed the ε_{max} values of all the cinnamoyl-CoA derivatives which he synthesized by comparing the extinction in the long wavelength UV region with the hydroxamate assay and used an extinction coefficient of cinna-

moyl hydroxamate-$FeCl_3$ complex as standard. He determined this by using an average value of his series of phenyl thiol esters prepared by hydroxylaminolysis.

While Gross and Zenk [3] reported an extinction coefficient for the cinnamoyl hydroxamate-iron complex of 1.54×10^6 cm²/mol [this value is not a missprint and not $\times 10^3$ too high as Johns [13] suggested but represents the molar extinction coefficient as widely used in biochemical literature], Johns [13] determined this coefficient as being 1.05×10^6 cm²/mol from his experiments. This value he also claimed to be more in line with that found for the acetyl-hydroxamic-iron complex [0.975×10^6]. This serious discrepancy led us to redetermine our previously published value. Analytically pure cinnamoyl hydroxamic acid was synthesized and samples dissolved in MeOH, at a concentration of 2 μmol/ml (25 ml total). Samples of 0.5 ml were removed and assayed as described by Johns [13]. An average value from 10 independent determinations was 1.68×10^6 cm²/mol at 540 nm. This corresponds to an extinction of 0.559 for 1 μmol cinnamoyl hydroxamate in 3 ml solvent. This value is even higher than that previously published [3] from this laboratory and proves that the extinction coefficient determined by Johns [13] is incorrect by more than one third.

The reason for this lower extinction value found by Johns [13] was most likely due to incomplete hydroxylaminolysis of the purified phenyl thiol esters, since the amount of hydroxylamine used (0.1 M final concentration) at pH 8.0 was relatively low. Kinetic experiments were therefore performed to study the conversion of the phenyl thiol ester of cinnamic acid (1 mg) and *p*-coumaroyl-CoA (0.5 μmol) to the corresponding hydroxamates under Johns [13] conditions. It was immediately seen that under these conditions hydroxamate formation of both thiol esters is incomplete. At the time interval (10 min) used by Johns [13] only about 30% of the thiophenyl ester was converted to the hydroxamate and after 25 min 50% was lysed. A complete conversion of 1 mg cinnamoyl thiophenyl ester to cinnamoyl hydroxamate under the given conditions [13], and within 10 min, was achieved only using salt free hydroxylamine. Only under these conditions was the theoretical extinction coefficient of the hydroxamate-iron complex reached. Using 1 M hydroxylamine (final concentration) at pH 8.0 the conversion was only 85% after 10 min. In the case of *p*-coumaroyl-CoA, 40% of the thiol ester present was transformed into the hydroxamate within 10 min as revealed by the colored iron complex, showing that the CoA derivative is somewhat more reactive under these conditions. Different rates of hydroxylaminolysis for different cinnamoyl CoA esters had been observed before [3]. Assuming a 10-fold half-life time for the complete conversion of *p*-coumaroyl-CoA to *p*-coumaroyl hydroxamate (1 M NH_2OH; pH 7.5; 20 °C) [3] this reaction would have taken 40 min to go to completion. Previously published data [3] had already shown that hydroxylaminolysis may be rate limiting.

No mention was made by Johns [13] of the fact that the *o*-dihydroxy groups of the caffeoyl moiety in the parent acid, as well as in caffeoyl phenyl thiol ester and caffeoyl-CoA, gives a pronounced color effect at 550 nm with the iron chloride reagent used in the absence of hydroxylamine. Accordingly, the calculated value [13] for caffeoyl-CoA is bond to be incorrect for this reason alone.

The fact that there was no quantitative conversion of the thiol esters to the hydroxamates, and that the rate of hydroxylaminolysis of the phenyl and CoA thiol esters was different, demonstrates beyond any doubt that Johns [13] method of calibrating the hydroxamate test for the determination of the ε_{max} values of acyl CoA thiol esters ist not accurate. Since the extinction coefficient of the cinnamoyl hydroxamate-iron complex used in the above method was too low, the reported ε_{max} values of the CoA derivatives are expected to be too high. This is indeed the case. We have redetermined the ε_{max} values [3] using 2-^{14}C-labelled cinnamic acids of known specific activity. By comparing the extinction at λ_{max} in the long UV region with the specific activity of the labelled CoA derivative a calculation of the ε_{max} values was possible. The ε_{max} value was counterchecked by using the enzymatic method of determining the molar extinction coefficient [3] of cinnamic acid, using hydroxycinnamoyl-CoA ligase [19] rather than acyl-CoA synthetase (EC 6.2.1.2.) from beef liver mitochondria. In order to make this reaction irreversible, pyrophosphatase (1 U) was included in the assay. Table IV shows the extinction coefficient values for the hydroxycinnamoyl-CoA thiol esters, their λ_{max} and λ_{min} values in the long wavelength UV region. These data are average values of between 8 to 10 individual determinations for each compound. No ε_{max} value for sinapoyl-CoA can be given, since a countercheck by the enzymatic

method was not possible due to the inability of hydroxycinnamoyl-CoA ligase to activate sinapic acid [19].

Table IV. UV-Absorption data for hydroxycinnamyol-CoA derivatives (measured in 0.1 M phosphate buffer pH 7.0).

CoA derivatives	λ_{max} I [nm]	λ_{max} II [nm]	λ_{min} [nm]	$\varepsilon_{max} \times 10^6$ at λ_{max} II [cm²/mol]
p-Coumaroyl	261	333	285	21
Caffeoyl	257	346	287	18
Feruloyl	257	346	287	19
Sinapoyl	252	352	289	—

As can be seen from the data given in Table IV, these values agree reasonably well with the values previously published from our laboratory [3]. One exception is caffeoyl-CoA, where both the λ_{max} and ε_{max} values of our previous determination is incorrect; this error had been noticed before [10]. While the λ_{max} values of the different hydroxycinnamoyl-CoA derivatives of Table IV agree with the ones reported by Johns [13], his measured ε_{max} values are too high as theoretically expected because of his erroneous calibration procedure. For example his published ε_{max} value for *p*-coumaroyl-CoA is about 1/3 too high (35×10^6) about the same order of magnitude by which his extinction coefficient of the cinnamoyl hydroxamate-iron complex is too low. Johns [13] statement that his ε_{max} values superceded the previously published ones [3] is therefore not tenable. Besides, if optical properties of CoA esters which are synthesized in two different laboratories are compared (Table IV, ref. 13), these determinations should be made at the same pH value (Johns [13]: pH 2.5 – 3.0; Gross and Zenk: pH 7.0). At any event, the difference in pH values used was not the reason for the difference in ε_{max} values. Furthermore missprints of quotations, *i. e.* λ_{max} values of *p*-coumaroyl-CoA as 351 nm [13] instead of 333 nm [3] should be avoided.

In addition Johns [13] has taken it as an encouraging sign of the reliability of his ε_{max} values for CoA thiol esters that they were closely similar to those of the equivalent phenyl thiol esters. We have redetermined the ε_{max} value of cinnamoyl phenyl thiol ester for which he reported an ε_{max} of 14.4×10^6 cm²/mol. As an average value from 6 independent determinations of the analytical pure compound we found 25.2×10^6 cm²/mol.

The excellent technical assistance of Miss B. Ries and Mr. L. Andert is gratefully appreciated. Financial support was provided by the "Deutsche Forschungsgemeinschaft", our thanks are due to Prof. J. McClure for his help in the english version of the manuscript and to Dr. G. G. Gross and Mr. W. Kreiten for adapting the column chromatography method [23] to cinnamoyl-CoA derivatives.

[1] A. J. Birch and F. W. Donovan, Austr. J. Chem. **6**, 360 [1953].
[2] H. Grisebach, Proceedings of the IVth International Congress of Biochemistry, Wien 1958, **Vol. II**, p. 56, Pergamon Press, London 1959.
[3] G. G. Gross and M. H. Zenk, Z. Naturforsch. **21 b**, 683 [1966].
[4] G. Billek and F. P. Schmook, Oesterr. Chem. Ztg. **67**, 401 [1966].
[5] M. H. Zenk, Proceedings of the 2nd Meeting of the Federation of Europ. Biochem. Societies, **Vol. 3**, p. 45 (G. Billek, ed.), Pergamon Press, London 1966. — M. H. Zenk, Ber. dtsch. Bot. Ges. **80**, 573 [1968].
[6] F. Kreuzaler and K. Hahlbrock, FEBS Lett. **28**, 69 [1972].
[7] R. L. Mansell, J. Stöckigt, and M. H. Zenk, Z. Pflanzenphysiol. **68**, 286 [1972].
[8] J. Ebel and H. Grisebach, FEBS Lett. **30**, 141 [1973].
[9] G. G. Gross, J. Stöckigt, R. L. Mansell, and M. H. Zenk, FEBS Lett. **31**, 283 [1973].
[10] J. Stöckigt and M. H. Zenk, FEBS Lett. **42**, 131 [1974].
[11] T. Lindl, F. Kreuzaler, and K. Hahlbrock, Biochim. Biophys. Acta **302**, 457 [1973].
[12] K. Hahlbrock, Ph. D. thesis, Freiburg 1965.
[13] N. Johns, Z. Naturforsch. **29 c**, 469 [1974].
[14] T. Wieland and L. Rueff, Angew. Chem. **65**, 186 [1953].
[15] E. G. Trams and R. O. Brady, J. Amer. Chem. Soc. **82**, 2972 [1960].
[16] J. C. Howard, M. C. Lin, P. M. Matthews, and S. A. Singal, J. Med. Chem. **8**, 888 [1965].
[17] H. Tanaka and A. Yokoyama, Chem. Pharm. Bull. (Tokyo) **10**, 13 [1962].
[18] W. Fuchs, Chem. Ber. **88**, 1825 [1955].
[19] G. G. Gross and M. H. Zenk, Eur. J. Biochem. **42**, 453 [1974].
[20] E. R. Stadtman, Methods in Enzymology, **Vol. III**, p. 931, Academic Press, New York 1957.
[21] F. Lipman and L. C. Tuttle, J. Biol. Chem. **159**, 21 [1945].
[22] C. R. Hauser and W. B. Renfrow, Organic Syntheses, Coll. **Vol. 2**, p. 67, A. H. Blatt, ed., John Wiley and Sons, New York 1963.
[23] C. Cha and R. E. Parks, J. Biol. Chem. **239**, 1961 [1964].
[24] A. Al-Arif and M. Blecher, J. Lipid Res. **10**, 344 [1969].
[25] M. E. Pullman, Anal. Biochem. **54**, 188 [1973].
[26] J. Ebel, B. Schaller-Hekeler, K.-H. Knobloch, E. Wellmann, H. Grisebach, and K. Hahlbrock, Biochim. Biophys. Acta **362**, 417 [1974].
[27] E. Walton and V. S. Butt, J. Exp. Bot. **21**, 887 [1970]; E. Walton and V. S. Butt, Phytochemistry **10**, 295 [1971]; M. J. C. Rhodes and L. S. C. Wooltorton, Phytochemistry **12**, 2381 [1973].
[28] E. Wellmann, D. Baron, and H. Grisebach, Biochim. Biophys. Acta **244**, 1 [1971].