white crystals slowly darkened in color between 90 and 120° and decomposed to a tarry mass between 120 and 125°. About 2 mg. of the octadecyl analog was subjected to phospholipase C incubation for 24 hr. (6), and the products were checked for the presence of 1-*O*-alkyldihydroxyacetone. However, the only product produced was a small quantity of fatty alcohol. Thus, it appears that 1-*O*-alkyl-3-*O*-phosphorylethanolamine-2-propanones, like the 1-*O*-alkyldihydroxyacetone phosphate, cannot serve as a substrate for phospholipase C. IR spectra revealed a strong carbonyl band at 1750 $cm.^{-1}$ in the regenerated ketone compounds, and this was completely absent in the dimethyl ketal derivatives (Fig. 3). The analyses are in agreement with those that would be expected from Structures IV and V illustrated in Scheme I.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 14, 1971, from the **Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514*, and the †*Medical Division of Oak Ridge Associated Universities, Oak Ridge, TN 37830*

Accepted for publication January 5, 1972.

Supported by National Institutes of Health Research Grant AM15172-07 from the U. S. Public Health Service and the U. S. Atomic Energy Commission.

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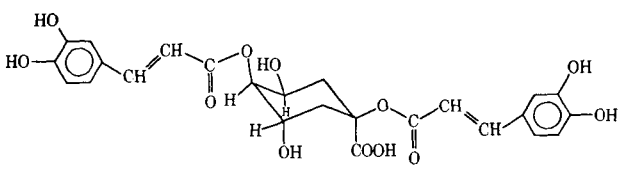
Dicinnamides as Potential Hypocholesterolemic Agents

ROY E. ALTMAN, Jr.*, and IRWIN L. HONIGBERG▲

Abstract □ A series of 12 alkyl and aryl dicinnamides related to cynarin were synthesized by the mixed anhydride method. However, attempts to synthesize these aromatic amides *via* the usual carbodiimide coupling methods were unsuccessful. These compounds were tested for their cholesterol-lowering properties in rats maintained on normal lab chow. No hypocholesterolemic effect was noted with the parent substance, cynarin, or any of its structural analogs.

Keyphrases □ Dicinnamides—synthesis, potential hypocholesterolemic agents □ Cinnamides, di—synthesis, potential hypocholesterolemic agents □ Hypocholesterolemic agents, potential—dicinnamides □ Structure-activity relationships—dicinnamides and hypocholesterolemic activity

In 1958, Preziosi and Loscalzo (1) performed a series of pharmacological tests on cynarin (Structure I), a compound isolated from artichokes (*Cynara scolymus*) and synthesized 4 years earlier (2). In these tests, it was

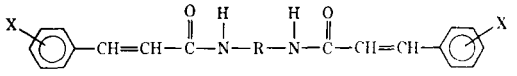


I

reported that the compound had a marked cholesterol-lowering effect on animals with induced hypercholesterolemia. In 1961, Mancini *et al.* (3) tested the effects of cynarin on serum cholesterol and lipoproteins in atherosclerotic patients. They reported: "In every case the administration of high doses of cynarin was followed by a decrease in total cholesterol and β-lipoprotein cholesterol. The α-lipoprotein cholesterol levels remained unchanged."

The drug is active only in high doses, and the duration of action is relatively short. Cynarin is a diester, and it is reasonable to expect the compound to be highly susceptible to hydrolysis by esterases in blood and tissue fluid. This degradation of cynarin by esterases can explain the need for the high doses required for biological activity. The present investigation is an attempt to explore the substitution of an amide linkage for the ester linkage in a series of model compounds related to cynarin. The increased stability of amides to enzymatic breakdown was described by Bloom and Laubach (4), who compared the prolonged duration of procaine amide to procaine. A molecule more potent than cynarin is desirable to reduce the size and frequency of administration without increasing the side effects. Various substituents on the cinnamic acid portions of the molecule also will be tried in an attempt to increase drug

Table I—Alkyl and Aryl Dicinnamides



Compound Number	X	R	Melting Point	Yield, %	Formula		Analysis, % Calc.	Analysis, % Found	IR, μ NH	IR, μ Amide I	IR, μ C=C	UV, nm. Amide II	UV, nm. $\lambda_{max.}$	UV, nm. $\epsilon \times 10^4$
1	H	$-(CH_2)_4-$	249–253°	60	$C_{22}H_{24}N_2O_2$	C	75.83	75.88	3.04	6.05	6.20	6.44	275	4.50
						H	6.94	7.02						
						N	8.04	8.11						
2	H	$-(CH_2)_2-$	243–244°	59	$C_{20}H_{20}N_2O_2$	C	74.98	74.91	3.04	6.07	6.18	6.48	276	5.04
						H	6.29	6.28						
						N	8.74	8.88						
3	H	*p*-C_6H_4	360° dec.	36	$C_{24}H_{20}N_2O_2$	C	78.24	78.18	3.09	6.01	6.15	6.40	279; 342	2.96; 3.40
						H	5.47	5.70						
						N	7.60	7.84						
4	OH	$-(CH_2)_4-$	251–253°	47	$C_{22}H_{24}N_2O_4$	C	69.46	69.42	3.01[a]	6.05	6.20	6.50	292	4.52
						H	6.35	6.26						
						N	7.36	7.25						
5	OCH_3	$-(CH_2)_4-$	229–231°	46	$C_{24}H_{28}N_2O_4$	C	70.57	70.44	3.05	6.05	6.21	6.50	293	5.18
						H	6.91	6.84						
						N	6.86	6.94						
6	CH_3	$-(CH_2)_4-$	250–252°	52	$C_{24}H_{28}N_2O_2$	C	76.56	76.61	3.05	6.05	6.20	6.44	281	4.86
						H	7.50	7.46						
						N	7.44	7.59						
7	NO_2	$-(CH_2)_4-$	278–280° dec.	65	$C_{22}H_{22}N_4O_6$	C	60.27	60.18	3.05	6.05	6.18	6.45[b]	321	3.81
						H	5.06	5.11						
						N	12.78	12.73						
8	Cl	$-(CH_2)_4-$	278–281° dec.	54	$C_{22}H_{22}Cl_2N_2O_2$	C	63.32	63.31	3.05	6.10	6.20	6.50	278	5.04
						H	5.31	5.35						
						N	6.71	6.86						
9	OCH_3	$-(CH_2)_4-CH(COOCH_3)-$	134–138°	57	$C_{27}H_{32}N_2O_6$	C	67.48	67.21	3.06[c]	6.08	6.22	6.50	292	5.28
						H	6.71	6.81						
						N	5.83	6.00						
10	CH_3	$-(CH_2)_4-CH(COOCH_3)-$	193–196°	58	$C_{27}H_{32}N_2O_4$	C	72.30	72.19	3.05[c]	6.03	6.18	6.50	285	5.31
						H	7.19	7.09						
						N	6.25	6.40						
11	NO_2	$-(CH_2)_4-CH(COOCH_3)-$	202–204°	54	$C_{25}H_{26}N_4O_8$	C	58.82	58.56	3.04[d]	6.04; 6.08	6.11; 6.18	6.50 6.60	305	4.49
						H	5.13	5.09						
						N	10.97	11.13						
12	Cl	$-(CH_2)_4-CH(COOCH_3)-$	208–210°	40[e]	$C_{25}H_{26}Cl_2N_2O_4$	C	61.36	61.21	3.05[c]	6.02	6.19	6.51	279	6.08
						H	5.36	5.41						
						N	5.72	5.51						

[a] OH band at 2.94. [b] NO_2 band at 6.55. [c] Ester band at 5.75. [d] Ester band at 5.79. [e] Estimate; entire product was not separated with preparative layer chromatography.

potency. Although these modifications are fundamental, they should not be overlooked in the search for better drugs to control abnormal cholesterol levels in individuals susceptible to atherosclerosis.

CHEMISTRY

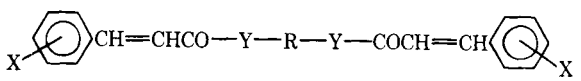
A general representation of the structure of cynarin is given in Structure II. The parent molecule is represented by X = 3,4-dihydroxy, R = 1,4-disubstituted quinic acid, and Y = oxygen.

In this investigation, Y = NH and X = a series of *para*-substituents. R, in this report, is ethylene, butylene, *p*-phenylene, and 1-carboxymethylbutylene. The cyclohexylene derivatives are currently under investigation and will be reported later.

The methods selected for the synthesis of the diamide analogs are based on procedures previously described in the literature for the formation of polypeptides (Scheme I).

Two methods were investigated: (*a*) the reaction of a mixed carbonic–carboxylic acid anhydride with an amine (5, 6), and (*b*) the treatment of a carboxylic acid and an amine with *N,N'*-dicyclohexylcarbodiimide as a coupling agent (7).

In the synthesis scheme the carboxyl component is a suitably substituted cinnamic acid with various electron-withdrawing or electron-donating groups. A series of diamino alkanes serves as the amine component. The compounds prepared are listed in Table I.



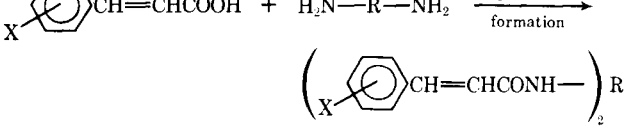
II

The diamide structure was confirmed by elemental analysis and by molar absorptivity, which is additive for the cinnamoyl moiety. The presence of the monoacyl urea was confirmed by IR spectra.

PHARMACOLOGY

The first attempts to develop a screening method for the cholesterol-lowering effect of the alkyl dicinnamides were based on the production of a hypercholesterolemic condition in the rat by the sublingual intravenous injection of tyloxapol[1]. This was then followed by injection of the drug (8). This procedure was abandoned when wide fluctuation in the initial hypercholesterolemia was observed, probably due to a variability in biological response to this material.

The method finally chosen is based on a 7-day feeding schedule of the drug to rats maintained on a normal lab chow diet. The procedure is as follows. Male Long-Evans rats, weighing approximately 100 g., were maintained on a diet of lab chow (Purina) and water *ad libitum*. The compounds to be tested were suspended in olive oil at 37° and administered intragastrically by intubation daily for 7 con-



Scheme I

[1] Triton WR 1339, Ruger Chemical Co., Irvington, NY 10533

Table II—Effect of Alkyl Dicinnamides on Serum Cholesterol

Compound Number	Dose, μm./ml.	Number of Animals	Percent Initial Serum Cholesterol
Controls	—[a]	4	97.95 ± 1.19[b]
Cynarin	40	4	138.4 ± 8.65[c]
AY-9944[d]	20	4	48.50 ± 3.70[c]
1	40	3	107.87 ± 0.74[c]
2	40	3	111.33 ± 4.85[c]
5	40	3	113.66 ± 5.89[c]
6	40	3	106.40 ± 3.37[c]
7	40	3	117.72 ± 10.26
8	40	3	118.62 ± 10.68

[a] Injected only with matched volume of suspending agent (olive oil). [b] Standard error. [c] $p = 0.05$. [d] *trans*-1,4-Bis(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride, supplied by Ayerst Laboratories.

secutive days. Control animals received an equal volume of olive oil on the same days. Prior to drug administration (Day 1) and 3 hr. following the final dose (Day 7), the rats were anesthetized with ether and 1-ml. samples of blood were removed by cardiac puncture. The blood samples were allowed to clot at room temperature for 1 hr. and were centrifuged twice to collect the serum. The total cholesterol concentration of the serum thus obtained was determined according to the method of Zlatkis *et al.* (9).

EXPERIMENTAL[2]

Arylene and Alkylene Cinnamides (Compounds 1-8, Table I)—The mixed anhydride method described by Vaughan and Osato (5, 6) was adapted to prepare the compounds listed in Table I. A solution of 0.05 mole of the proper cinnamic acid and 5.08 g. (0.05 mole) of triethylamine in 150 ml. of chloroform was cooled to −5° in a salt–ice bath, and this temperature was maintained throughout the reaction. To this solution, 6.83 g. (0.05 mole) of isobutylchlorocarbonate in 50 ml. of chloroform was added. The reaction mixture was allowed to stir 25 min. to allow completion of the mixed anhydride formation. A solution of 0.025 mole of the desired diamino compound in 30 ml. of chloroform was then added. After addition, the reaction mixture was allowed to warm to room temperature and was stirred overnight. The crude product, which precipitated from the reaction mixture, was removed by filtration and washed with water, 5% hydrochloric acid, 5% potassium hydroxide, and again with water. Dimethylformamide–water was the crystallization solvent for all of the products.

After the initial filtration, the reaction solvent was washed in succession with water, acid, and base. An oily residue, which could not be crystallized, remained after evaporation of the chloroform.

1,4-Butylene-*p*-hydroxycinnamide (Compound 4, Table I)—A solution of 8.21 g. (0.05 mole) of *p*-hydroxycinnamic acid and 10.1 g. (0.10 mole) of triethylamine in 150 ml. of chloroform was cooled to −5°. To this cold solution, 13.66 g. (0.10 mole)[3] of isobutylchlorocarbonate was added, and the reaction was allowed to stir for 20 min., after which time 2.21 g. (0.025 mole) of putrescine was added. The reaction mixture was then allowed to warm to room temperature and was stirred overnight. Very little solid was present, which indicated the *O*-protected intermediate was in solution. The reaction mixture was filtered, and the filtrate was washed successively with water, 5% hydrochloric acid, 5% sodium bicarbonate, and finally with water. After drying the mixture over sodium sulfate, the chloroform was evaporated to dryness *in vacuo*.

The residue was recrystallized from ethanol–water to yield 8.9 g. (61%) of *O*-protected intermediate, m.p. 232–235°. The IR spectrum showed the bands (3.0 and 6.4) characteristic for the diamides and a band at 5.65 (ester).

The 8.9 g. of *O*-protected intermediate and 100 ml. of 10% potassium carbonate (20 ml. of ethanol was added to effect solution of the solid) were boiled in a beaker for 36 hr., during which time the ethanol evaporated. Upon cooling, the 1,4-butylene-*p*-hydroxycinnamide crystallized. An additional 0.5 g. was obtained when the basic solution was acidified with hydrochloric acid. The combined products were recrystallized from ethanol–water after decolorizing with activated charcoal[4].

Methyl *N,N'*-Bis(cinnamoyl)lysinates (Compounds 9-11, Table I)—The mixed anhydride was prepared by the procedure given in the synthesis of 1,4-butylene cinnamides. A solution of 4.0 g. (0.025 mole) of methyl lysinate (11) in 50 ml. of chloroform was added slowly to the reaction mixture. The mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was washed in a separator with 5% potassium hydroxide, 5% hydrochloric acid, and three 50-ml. portions of water. After the mixture was dried over sodium sulfate, the chloroform was evaporated *in vacuo*. The residual oil obtained on evaporation of the chloroform was suspended in ethyl acetate, from which a solid precipitated on standing. The solid mass was washed with hexane and recrystallized from ethyl acetate–hexane.

Methyl *N,N'*-Bis(*p*-chlorocinnamoyl)lysinate (Compound 12, Table I)—This compound was prepared by the procedure given for Compounds 9–11. The solid residue obtained on evaporation of the chloroform was dissolved in hot methanol and reprecipitated by the addition of water (67% yield of crude product). TLC on silica gel HF_{254}, using ethyl acetate–hexane (9:1) as the developing solvent, indicated three compounds to be present in the crude product. The R_f values of the impurities were 0.33 and 0.26, which were less than the product R_f value of 0.49. This indicated that the impurities are more polar than the major product, methyl *N,N'*-bis(*p*-chlorocinnamoyl)lysinate. The data are consistent with the interpretation that the impurities are believed to be the monacylated lysinates, although these bands were not isolated. A product suitable for analysis was obtained with the use of preparative layer chromatography.

Attempted Preparation of *N,N'*-Dibenzoyl Putrescine—To a solution of 2.06 g. (0.01 mole) of *N,N'*-dicyclohexylcarbodiimide and 1.22 g. (0.01 mole) of benzoic acid in methylene chloride was added at once 0.44 g. (0.005 mole) of putrescine (1,4-butanediamine). A precipitate formed immediately and the reaction mixture was allowed to stir for 5 hr. The solid mass was identified as the byproduct, *N*-benzoyl-*N,N'*-dicyclohexylurea (2.7 g., 82.5%), m.p. 160–161° [lit. (10) m.p. 160–161°]. The filtrate from the reaction was washed with 5% hydrochloric acid, 5% sodium bicarbonate, and water. A small quantity of oil remained after the washed filtrate was dried over sodium sulfate and evaporated *in vacuo*. Attempts to crystallize the oil were unsuccessful.

Both the order of addition of the reagents and the concentration of the reagents in the solvent system were varied but yielded the acyl urea each time, with only a single exception—*viz.*, when the benzoic acid was added to a mixture of *N,N'*-dicyclohexylcarbodiimide and putrescine in methylene chloride, putrescine benzoate was formed in quantitative yield. This precipitate was characterized by: (*a*) its ready solubility in water and (*b*) precipitation of benzoic acid on acidification.

RESULTS AND DISCUSSION

The synthesis of the alkyl and aryl diamides proceeded quite satisfactorily with the use of the mixed anhydride method. It is not well documented that the usual carbodiimide coupling methods fail when used with the aromatic acids. A review of the literature revealed only one case in which dicyclohexylcarbodiimide was used successfully to activate the carboxyl group of an aromatic acid (12). A study of the conditions necessary for the coupling of aromatic acids with amines in the presence of this reagent is planned.

The pharmacological results (Table II) show that cynarin and the alkyl and aryl dicinnamides have no cholesterol-lowering effect in the present test system, although AY-9944, a compound known to inhibit cholesterol synthesis, has a marked effect in this system.

Autopsies of the animals 3 hr. after the last injection revealed a considerable amount of unabsorbed dicinnamides remaining in the GI tract. Poor absorption of the drugs also was evident from a close examination of the cage droppings during the screening program, in which large amounts of each drug were excreted in the feces. Cyna-

[2] Melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer model 202 spectrophotometer. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

[3] An excess (1 equivalent) of isobutylchlorocarbonate was added to protect the hydroxy group in *p*-hydroxycinnamic acid.

[4] Norit.

rin, a more polar molecule, showed no residue on autopsy, indicating complete absorption from the olive oil suspension.

It would be expected that compounds containing more polar groups, such as the dicinnamoyl lysines, would more clearly reveal any relationship between the alkylene and cycloalkylene diamides and the natural product, cynarin. The work is currently under study.

The lack of any cholesterol-lowering effect of cynarin in the present test system suggests that this group of compounds may only show their effectiveness in previously hypercholesterolized animals, as originally reported by Preziosi and Loscalzo (1).

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 15, 1969, from the *Department of Medicinal Chemistry, School of Pharmacy, University of Georgia, Athens, GA 30601*

Accepted for publication January 4, 1972.

Supported by Grant HE 09933 from the National Institutes of Health, U. S. Public Health Service, Bethesda, MD 20014

The authors thank Mrs. E. Garst and Mrs. K. Fields for technical assistance in performing the pharmacological tests. They also acknowledge the valuable consultations of Dr. A. E. Wade in developing the pharmacological tests.

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DRUG STANDARDS

Determination of Chloral Hydrate in Soft Gelatin Capsules by NMR

JOHN W. TURCZAN▲ and BRUCE A. GOLDWITZ

Abstract □ An accurate and specific procedure is described by which chloral hydrate is determined. Analysis of six synthetic mixtures showed that this method is accurate to ±1% with a standard deviation of 0.6. The analysis of commercial chloral hydrate capsules provides results that are within 1–2% by the NMR method, whereas the USP XVIII assay yields values that are, on the average, 6% higher than the labeled amount. It is felt that the high results obtained with the official procedure may be ascribed to the nonspecificity of the acid–base titration.

Keyphrases □ Chloral hydrate soft gelatin capsules—NMR analysis, compared to compendial method □ Capsules, chloral hydrate—NMR analysis, compared to compendial method □ NMR spectroscopy—analysis, chloral hydrate soft gelatin capsules

Published procedures for the analysis of chloral hydrate (2,2,2-trichloro-1,1-ethanediol) abound. Some are based on the Fujiwara color-producing reaction with alkaline pyridine (1–6). This reaction is nonspecific, since the red color may be produced by many polyhalogenated aliphatic compounds. Other procedures, such as the one by Stehwien and Kuhmstedt (7) which reacts chloral hydrate with 2,6-diaminopyridine, seem specific but critical conditions are required. The one developed by Archer and Haugas (8), based upon the reaction of chloral hydrate with quinaldine ethyl iodide to produce a stable blue cyanine dye, shows no interference from polyhalogenated compounds.

In addition to the colorimetric methods, other approaches have included GC, polarography, and titra-

Table I—Determination of Chloral Hydrate in Standard Mixtures by NMR[a]

Standard Mixture	Maleic Acid Internal Standard Added, mg.	Chloral Hydrate Added, mg.	Chloral Hydrate Found, mg.	Chloral Hydrate Recovery, %
1	126.0	505.1	508.6	100.7
2	176.9	507.9	510.0	100.4
3	228.1	505.9	505.9	100.0
4	182.6	524.4	524.9	100.1
5	180.5	519.8	514.6	99.0
6	177.6	504.1	501.1	99.4

[a] $SD = 0.6$.