Synthesis of 1-*O*-Alkyl-3-*O*-phosphorylethanolamine-2-propanone and Related Derivatives

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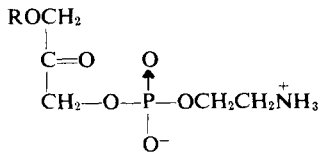
Abstract □ The synthesis and characterization of 1-*O*-hexadecyl- and 1-*O*-octadecyl-3-*O*-phosphorylethanolamine-2-propanone are described. Ketal derivatives of these compounds were isolated and purified by column chromatography on silica gel in an overall yield of 21 and 27%. The availability of these new keto phospholipids could be important in biosynthetic studies of alkyl glycerolipids, plasmalogens, and related derivatives.

Keyphrases □ 1-*O*-Alkyl-3-*O*-phosphorylethanolamine-2-propanones—synthesis, characterization, ketal derivatives □ 1-*O*-Hexadecyl-3-*O*-phosphorylethanolamine-2-propanone—synthesis, characterization, ketal derivative □ 1-*O*-Octadecyl-3-*O*-phosphorylethanolamine-2-propanone—synthesis, characterization, ketal derivative □ Keto phospholipids—synthesis, characterization □ Phospholipids, keto—synthesis, characterization

This paper describes the chemical synthesis of a new class of ether-linked keto phospholipids, 1-*O*-alkyl-3-*O*-phosphorylethanolamine-2-propanones:



Interest in these novel lipids and related derivatives is derived from studies by Snyder *et al.* (1–4) and Wykle and Snyder (5, 6) on the enzymic synthesis of *O*-alkyl ether bonds in glycerolipids. *O*-Alkyldihydroxyacetone phosphate is the first detectable intermediate formed by the microsomal enzyme system, which is also capable of synthesizing alkylacylglycerylphosphorylethanolamine and alkylacylglycerylphosphorylcholine (3, 6). Others, including Hajra (7, 8) and Kapoulas and Thompson (9), also demonstrated the presence of similar enzymic activities in other systems.

The enzymic studies led to the investigation of the chemical synthesis of 1-*O*-alkyl-3-*O*-phosphorylethanolamine-2-propanone and related analogs. In recent communications, Piantadosi *et al.* (10, 11) reported on the synthesis and characterization of 1-*O*-alkyldihydroxyacetone phosphates, the key starting material used to synthesize 1-*O*-alkyl-3-*O*-phosphorylethanolamine-2-propanones.

Table I—TLC Relative R_f Values of 1-*O*-Alkyl-3-*O*-phosphorylethanolamine-2-propanones and Derivatives on Thin-Layer Chromatograms[a]

Compound	Solvent 1[b]	Solvent 2	Solvent 3
Alkyldimethoxy GPE[c]	0.48	0.41	N.D.[d]
Alkyl keto GPE	0.40	0.29	0.13
Acyl lyso GPE	0.35	0.29	N.D.
Diacyl GPE	0.74	0.56	0.28
Diacyl GPC	0.26	0.40	0.15
DNB-alkyl keto GPE	N.D.	N.D.	0.44
DNB-alkyl lyso GPE	N.D.	N.D.	0.43
DNB-diacyl GPE	N.D.	N.D.	0.64

[a] Silica gel HR. [b] Solvent 1: chloroform–methanol–HAc–water, 50:25:8:2. Solvent 2: chloroform–methanol–NH_4OH, 65:35:8. Solvent 3: chloroform–methanol–NH_4OH, 70:30:2. [c] Abbreviations: GPE = glycerylphosphorylethanolamine, GPC = glycerylphosphorylcholine, and DNB = dinitrobenzene. [d] N.D. = not determined.

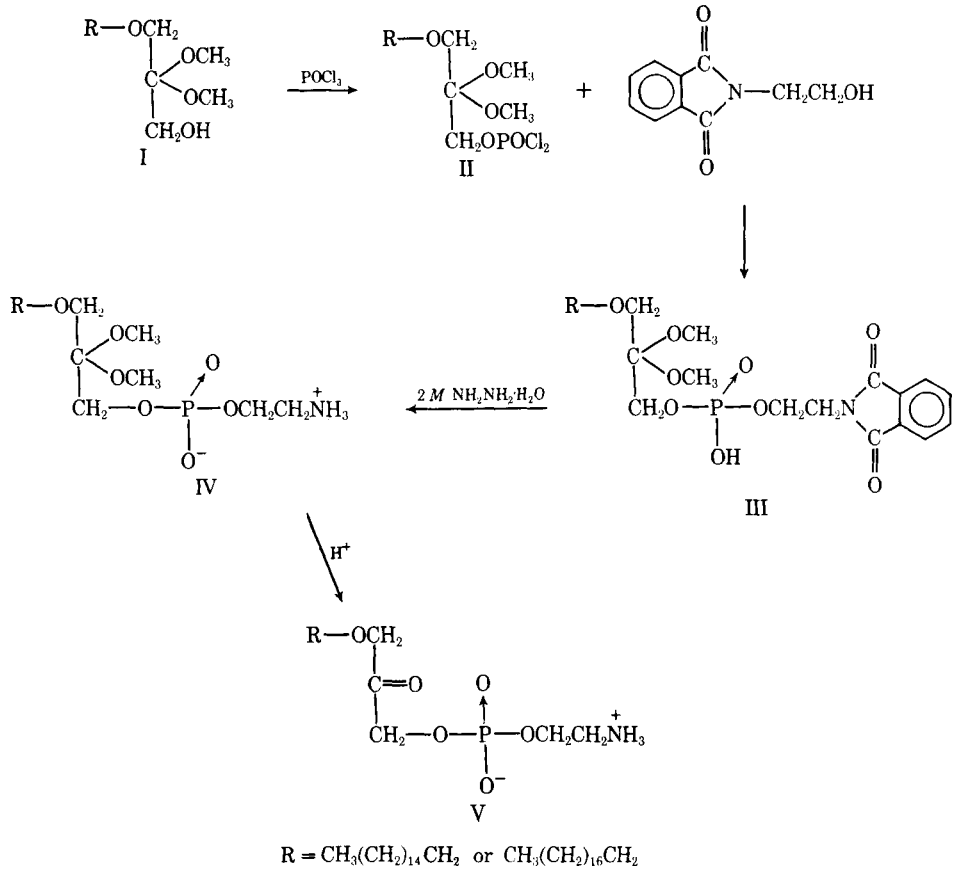
EXPERIMENTAL[1]

Synthesis and Characterization of IV: 1-*O*-Hexadecyl-2,2-dimethoxypropane-3-*O*-phosphorylethanolamine and 1-*O*-Octadecyl-2,2-dimethoxypropane-3-*O*-phosphorylethanolamine—The overall experimental method (Scheme I) was based on reactions previously described by Piantadosi *et al.* (11, 13) and Egerton and Malkin (14). Phosphorus oxychloride (3.6 g.) was dissolved in 15 ml. of $CHCl_3$ in a three-necked 250-ml. flask which was equipped with a magnetic bar, dropping funnel, thermometer, condensor, and drying tube containing anhydrous $CaCl_2$. The flask was chilled in an ice bath and stirred vigorously; when the temperature of the solution reached 0°, 9.5 ml. of pyridine was added dropwise within a period of 30 min. The temperature of the solution was then raised to 10°, and 7.7 g. of 1-*O*-hexadecyl-2,2-dimethoxy-3-hydroxypropane, dissolved in 30 ml. of $CHCl_3$ and 1 ml. of pyridine, was added dropwise with vigorous stirring at 10–12° over a period of 2 hr. The temperature of the solution was raised to 25° and maintained at this temperature for 0.5 hr. with stirring; the temperature of the solution was then increased to 40° and stirred for another 0.5 hr. Next, the reaction mixture was cooled to 12°, and 4.6 g. of *N*-2-hydroxyethylphthalimide, dissolved in 50 ml. of $CHCl_3$, was added dropwise over a 1-hr. period with stirring. The stirring was continued for another 0.5 hr. at 30°. The temperature of the reaction mixture was then raised to 47° and maintained for another 0.5 hr. The mixture was chilled to 0°, and 15 ml. of 10 *N* NaOH was added dropwise within 0.5 hr.

The resulting mixture was transferred to a separator; 50 ml. of $CHCl_3$ and 50 ml. of water were added and the separator was placed overnight in a cold room at 5°. The two phases that formed were separated and the solvents were removed under vacuum. The syrupy residue obtained was dissolved by warming in 75 ml. of 2-ethoxyethanol[2]. The solution was transferred to a 300-ml. round-bottom flask, and 5 ml. of 1 *N* NaOH and 20 ml. of 2 *M* hydrazine hydrate were added. This mixture was heated to 130–135° for 1 hr. with vigorous stirring on an oil bath and then stirred overnight at room temperature. The crude product (4.8 g.) that separated as a solid was filtered under vacuum, washed with 100 ml. of ice-cold acetone, and finally dried under vacuum in a desiccator over Drierite and P_2O_5. Another 1.3 g. of crude product was obtained from the mother liquor. 1-*O*-Octadecyl-2,2-dimethoxy-3-*O*-phos-

[1] All melting points were taken in a Mel-Temp melting-point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Garden City, Mich., and Atlantic Microlab Inc., Atlanta, Ga. IR spectra were determined with a Perkin-Elmer 257 spectrophotometer, and NMR spectra were obtained with a Varian HA-100 spectrometer. Silica gel 70-325 mesh was used for column chromatography; silica gel G and silica gel HR (the latter containing 0.001 *M* Na_2CO_3) were used for TLC (Brinkmann Instruments, Inc.). $POCl_3$ was freshly distilled immediately before use, pyridine was distilled after drying over KOH, and chloroform (ethanol was removed by washing with concentrated H_2SO_4 and then with water until free of acid) was distilled after drying over $CaCl_2$–K_2CO_3. The 1-*O*-alkyl-2,2-dimethoxy-3-hydroxypropane (11) used as the starting material was prepared by a modification of the method used by Hartman (12) for the preparation of haloacetol phosphates.

[2] Ethyl cellosolve.



Scheme I

phorylethanolamine [IV, where R = $CH_3(CH_2)_{16}CH_2$] was prepared in an analogous manner by starting with 7.5 g. of 1-*O*-octadecyl-2,2-dimethoxy-3-hydroxypropane. The yield was 6.7 g.

The products were purified by column chromatography. One gram of 1-*O*-alkyl-2,2-dimethoxypropane-3-*O*-phosphorylethanolamine was dissolved in a minimum amount of $CHCl_3$ and placed on columns (1.9-cm. diameter) containing 30 g. silica gel. Elutions were carried out first with 100 ml. of $CHCl_3$ and then with 150-ml. portions of various proportions of $CHCl_3$–CH_3OH (ratio in parentheses, v/v): Fraction I (9:1), Fraction II (8:2), Fraction III (7:3), Fraction IV (6:4), and Fraction V (5:5).

1-*O*-Hexadecyl-2,2-dimethoxypropane-3-*O*-phosphorylethanolamine [IV, where R = $CH_3(CH_2)_{14}CH_2$] began to appear in Fraction III, but the bulk was in Fraction IV. Fractions III, IV, and V were recycled on a 20-g. silica gel column using the same elution pattern. The fractions containing the product were combined and the solvent was removed; the solid residue was redissolved in 10 ml. of chloroform. It was suction filtered and the solvent was removed under nitrogen. The residual $CHCl_3$ was removed under vacuum in a desiccator over P_2O_5 and Drierite. The yield of product was 370 mg. (21% based on 1-*O*-hexadecyl-2,2-dimethoxy-3-hydroxypropane) and its melting point was 151.5–154° (decomposed with prior softening). TLC on silica gel G in solvent systems of $CHCl_3$–CH_3OH (8:2) and CH_3OH–$CHCl_3$–H_2O (25:65:4) revealed the presence of a single spot yielding a positive ninhydrin test. The IR spectrum (KBr), depicted in Fig. 1, is consistent with the proposed structure and shows the following absorption bands: CH_3 and CH_2 (2920, 2850, and 1465 cm.$^{-1}$), $-\overset{+}{N}H_3$ (1624 and

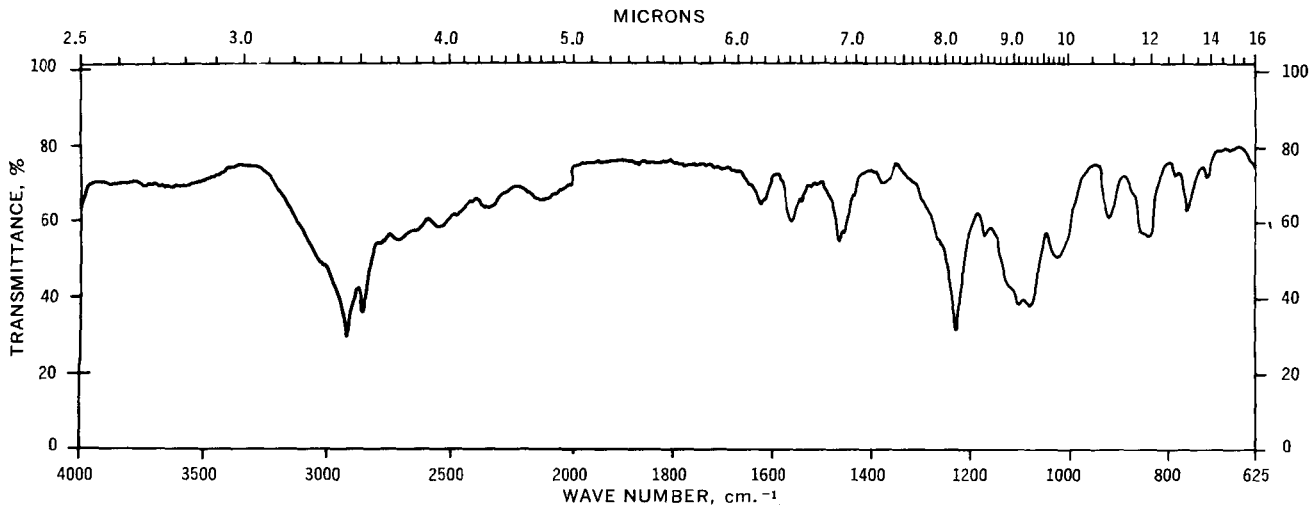


Figure 1—*IR spectrum of 1-O-hexadecyl-2,2-dimethoxypropane-3-O-phosphorylethanolamine.*

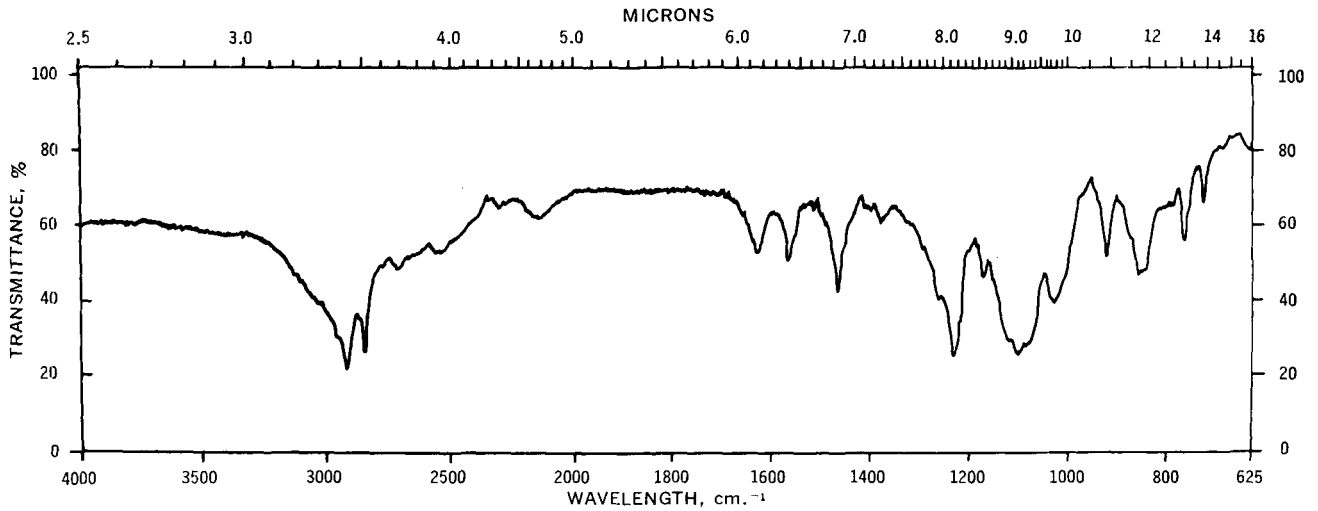


Figure 2—*IR spectrum of 1-O-octadecyl-2,2-dimethoxypropane-3-O-phosphorylethanolamine.*

1557 cm.$^{-1}$), P=O (1231 cm.$^{-1}$), and —C—O—C— (1150–1070 cm.$^{-1}$). NMR data: ($CDCl_3$) δ 0.89 (t, 3H, J = 7 Hz., CH_3), 1.25 (s, sharp, 28H, CH_2-long chain), and 3.23 (s, 6H OCH_3).

Anal.—Calc. for $C_{23}H_{50}NO_7P$: C, 57.11; H, 10.42; N, 2.89; P, 6.40. Found: C, 57.31; H, 10.59; N, 2.93; P, 6.35.

The yield of 1-*O*-octadecyl-2,2-dimethoxypropane-3-*O*-phosphorylethanolamine was 400 mg. (27% based on 1-*O*-octadecyl-2,2-dimethoxy-3-hydroxypropane); it had a melting point of 150–152° (decomposed with prior softening), and the ninhydrin test was positive. The IR spectrum of the octadecyl analog (KBr) is depicted in Fig. 2 and is consistent with the proposed structure. Furthermore, the absorption bands are analogous to the 1-*O*-hexadecyl-2,2-dimethoxypropane-3-*O*-phosphorylethanolamine.

Anal.—Calc. for $C_{25}H_{54}NO_7P$: C, 58.68; H, 10.63; N, 2.73; P, 6.05. Found: C, 58.44; H, 10.59; N, 2.71; P, 5.83.

Formation and Characterization of 1-*O*-Alkyl-3-*O*-phosphorylethanolaminepropanone—The ketal derivatives (10 mg.) were spread as a thin film on the inside of a 50-ml. pear flask and the flask was then filled with hydrochloric acid gas, stoppered, and allowed to stand for 1 hr. At the end of this period, no unreacted ketal derivatives remained. The main products visualized by TLC were the alkylphosphorylethanolaminepropanones and some fatty alcohols, the latter being a breakdown product. Previously it was observed that alkyldihydroxyacetone phosphate was not stable when exposed to rigorous acid hydrolysis (3).

The total reaction mixture was transferred with $CHCl_3$ to a 15-ml. column centrifuge tube and the $CHCl_3$ was removed with a stream of N_2. Ten milliliters of anhydrous diethyl ether were added and the contents were shaken vigorously for about 2 min.; the portion of the sample that did not dissolve was isolated by centrifugation. The supernate contained the fatty alcohols and other unidentified breakdown products. The insoluble material was later identified as 1-*O*-alkyl-3-*O*-phosphorylethanolaminepropanones. For these studies, the insoluble material was washed a second time with 5 ml. of diethyl ether. The residue, isolated by centrifugation, was dissolved in 4 ml. of $CHCl_3$; then 4 ml. of methanol and 4 ml. of water were added, mixed, and centrifuged. The chloroform layer and chloroform extracts of the water layer contained the 1-*O*-alkyl-3-*O*-phosphorylethanolaminepropanones (about 50% yield) as determined by the chromatographic, chemical, and IR analyses described in the remainder of this section.

Migration of the 1-*O*-alkyl-3-*O*-phosphorylethanolamine-2-propanones and a number of standard reference compounds was compared on silica gel HR layers in several solvent systems (Table I). In general, their chromatographic properties are similar to those of lysophosphatidylethanolamine. This is also borne out by the behavior of the dinitrobenzene derivatives (15).

The 1-*O*-alkyl-3-*O*-phosphorylethanolamine-2-propanones yielded 1-*O*-alkylglycerols after Vitride [$NaAlH_2(OCH_2CH_2OCH_3)_2$] reduction (16), and these were identified by TLC (17) and by GLC of their isopropylidene derivatives (18). The TLC R_f's of the *O*-alkylglycerols and their isopropylidene derivatives were identical to those of authentic hexadecylglycerol and octadecylglycerol. GLC analysis of the chain-length distribution in the *O*-alkylglycerol derived from the hexadecyl and octadecyl analogs demonstrated that the former contained 77.8% of the 16:0 chains and that the latter contained 91.4% of the 18:0 chains. Contamination of other chain lengths was due to impurities in the natural *O*-alkylglycerols used to synthesize the 1-*O*-alkyl-2,2-dimethoxy-3-hydroxypropane.

An attempt was made to determine the melting point for the 1-*O*-octadecyl-3-*O*-phosphorylethanolamine-2-propanones, but the

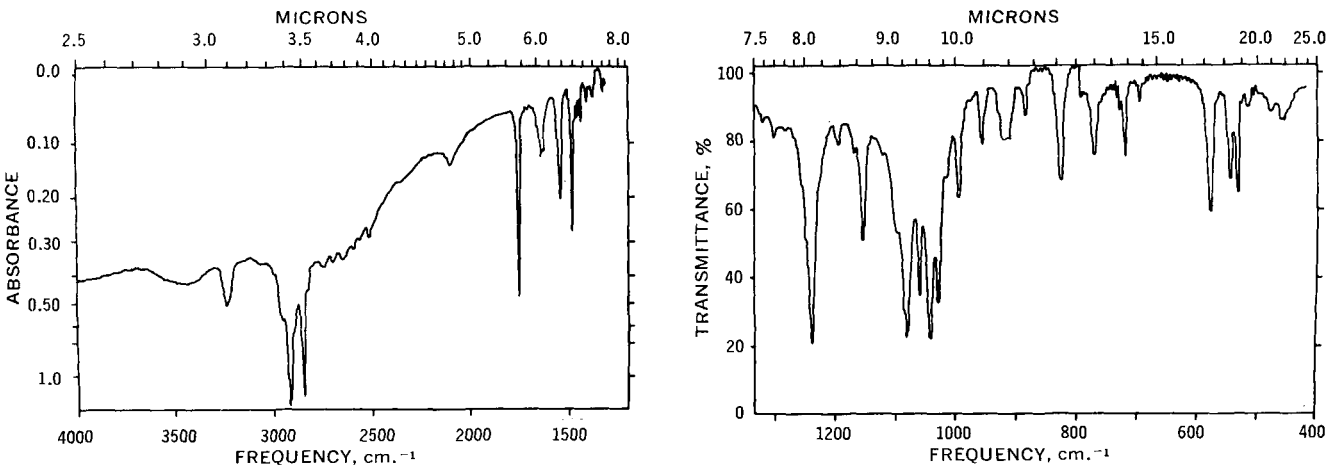


Figure 3—*IR spectra of 1-O-hexadecyl-2-propanone-3-O-phosphorylethanolamine.*

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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Dicinnamides as Potential Hypocholesterolemic Agents

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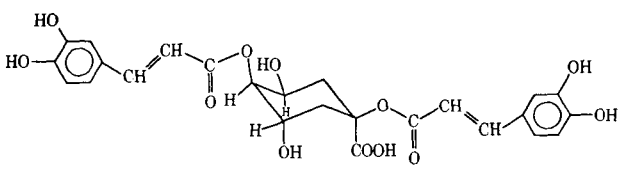
Abstract □ A series of 12 alkyl and aryl dicinnamides related to cynarin were synthesized by the mixed anhydride method. However, attempts to synthesize these aromatic amides *via* the usual carbodiimide coupling methods were unsuccessful. These compounds were tested for their cholesterol-lowering properties 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



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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