EXPERIMENTAL (BIOLOGY)

The extent of aggregation of thrombocytes was measured photometrically using Born's method, as described by Lakin [1], and recorded graphically by the changes in optical density of the thrombocyte-enriched plasma following introduction of Reanal grade ADP (Hungary) as an aggregation inducer at a final concentration of 10^{-3} mole/liter, on a Chronolog two-channel automatic aggregometer (USA), model 440. Thrombocyte-enriched plasma was obtained by prior centrifugation of sodium citrate-stabilized whole blood drawn from the ulnar vein of a donor or the abdominal aorta of nembutal-narcotized rats. The (I) hydrochloride was dissolved in 0.1 ml of isotonic sodium chloride solution in concentrations of 0.175 and 0.4 mg/ml, corresponding to the presence in the body of harmless doses of 1/5 and 1/3 of the LD_{50}. To 0.9 ml of TEP was added the (I) hydrochloride solution, and the mixture incubated for five minutes prior to measurement. The control, taken as 100%, was the minimum irreversible aggregation of thromobocytes in the presence of the inducer only in a sample containing 0.1 ml of isotonic saline solution per 1 ml of thrombocytic plasma.

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SYNTHESIS, AND HYPOLIPIDEMIC AND CHOLAGOGIC ACTIVITY OF CINNAMIC ACID DERIVATIVES

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There have been reports of high hypolipidemic and cholagogic activity in cinnamic acid derivatives [14, 22]. Examination of these reports leads to the following conclusions: 1) an essential requirement for activity is the presence of an α,β-unsaturated carbonyl fragment; 2) the introduction of hydroxy-, methoxy-, carboxy-, or alkoxycarbonyl groups into the 3 or 4-position of the benzene ring results in a marked increase in activity; 3) the presence of a dialkoxy group in the 3- or 4- position of the benzene ring reduces activity considerably, and 4) conversion of the carboxyl group into salts or esters does not have any significant effect on activity. The effects of cinnamic acids on various enzyme systems indicate that compounds possessing quite a small number of substituents can nevertheless exhibit a wide range of biological activity [17, 20, 23]. Reports of the hypolipidemic activity of thyroxine led to a search for analogs with respect to activity amongst aryloxyalkanoic acids, in order to identify antisclerotic drugs [3, 19, 21] such as misclerone. It has been reported in a review [8] that an invariable requirement for the display of this type of activity is the presence of a carboxy or alkoxycarbonyl group, which is probably responsible to the interaction of the molecule with enzymes (HMG reductase and acetylcarboxylase). According to our earlier work, hypolipidemic and cholagogic activity in a series of triterpene acids is due to the presence of a free carboxy group [5, 11]. In order to confirm this conclusion, and to assess the contribution of an alkoxycarbonyl group, the 3-carboxymethoxy derivative of oleanolic acid was prepared. The introduction of a carboxymethyl group led to a marked increase in hypolipidemic activity as compared with that of oleanolic acid itself.

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TABLE 1. Physicochemical Data for Compounds Obtained

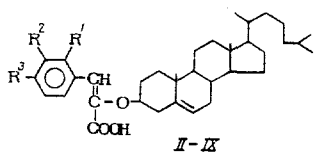
Compound	Mp, °C	Empirical formula	Control, zero	
			AP	IR, cm^{-1}
I	141	$C_{29}H_{48}O_3$	262	3400, 1760, 1470, 1380, 1100, 1060
II	98	$C_{36}H_{52}O_3$	247, 280	3400, 1720, 1470, 1380, 1210, 1040, 965
III	110	$C_{36}H_{52}O_4$	212, 271	3400, 1750, 1530, 1250, 1050, 970
IV	105	$C_{37}H_{52}O_6$	205, 231, 279	3400, 1720, 1680, 1470, 1380, 1250, 1050, 980
V	114	$C_{37}H_{54}O_5$	211, 277	3400, 1740, 1530, 1250, 1040, 965
VI	120	$C_{37}H_{54}O_5$	221, 269, 292	3400, 1740, 1530, 1240, 1065, 970
VII	106	$C_{36}H_{51}NO_5$	222, 270, 346	3400, 1740, 1530, 1230, 1035, 965
VIII	101	$C_{37}H_{54}O_4$	213, 280,	3400, 1750, 1535, 1245, 1155, 980
IX	109	$C_{37}H_{54}O_4$	206, 242	3400, 1750, 1540, 1240, 1150, 980

In patients with atherosclerosis, there is an increase in the content of bile acids in the blood, and a decrease in the bile, owing to the conversion of substantial amounts of cholesterol into bile acids, and inadequate excretion thereof in the bile as a result of the impairment of the secretory function of the liver [1, 7].

It was, therefore, of interest to examine the relationship between cholagogic and hypocholesteremic activity within groups of related compounds. We have already examined the cholagogic activity of ursolic and oleanolic acids and of glyciram, which show high hypocholesteremic activity. Our studies showed that triterpenes markedly increase bile excretion, and raise the cholate–cholesterol index [4]. These findings indicate that there is a link between cholesterol metabolism and the bile excretory function of the liver.

These considerations provide a rationale for a search for compounds which possess both cholagogic and hypocholesteremic activity. With this in mind, it was decided to synthesize some cinnamic acids bearing triterpenoid or steroid residues at the vinylene moiety. Carboxy an dcarboxyalkoxy groups considerable interest to separate these features.

To attain these objectives, cholesterol was used, this compound being employed in experimental biology to produce model arteriosclerosis [6]. Condensation of sodium cholesterate with chloroacetic acid gave 3-carboxymethyoxycholest-5-ene (I). This compound, which contains a CH_2-acid center, was used as the starting material for the synthesis of cholest-5-ene derivatives of cinnamic acids (Table 1).



II-IX

R^1=H(II—VI, IX), NO_2(VII), OCH_3(VIII);
R^2=H(II, III, VII—IX), COOH(IV), OCH_3(V), OH(VI); R^3=H(II, VII, VIII), OH(III—V), OCH_3(VI, IX).

Compounds (II-IX) were obtained by condensing (I) with aromatic aldehydes in acetic anhydride in the presence of triethylamine. The physicochemical data for the compounds are given in Table 1.

Tests have shown that these compounds possess both hypolipidemic and cholagogic activity, confirming that there is a relationship between cholesterol metabolism and the bile-excretory function of the liver (Tables 2 and 3). The greatest hypolipidemic activity was found in (I), exceeding that of misclerone, a hypocholesteremic drug. The effects of this compound on cholesterol and triglyceride levels varied according to the dose used, since when the dose was raised to 75 mg/kg the hypolipidemic activity increased, but the hypocholesteremic activity decreased.

TABLE 2. Hypolipidemic Activity of Compounds Obtained

Test conditions	n	Blood cholesterol, mmole/liter			Blood triglycerides, mmole/liter		
		M±m		P	M±m		P
Untreated animals	10	1,846±0,052		—	0,715±0,044		—
Hyperlipidemic animals (controls)	6	7,150±0,156		—	8,778±0,088		—
Hyperlipidemic animals receiving:							
I, 10 mg/kg	6	4,550±0,336	(−36,0)	<0,01	7,590±0,187	(−27,0)	<0,01
I, 75 mg/kg	6	1,746±0,260	(−20,0)	<0,01	6,688±0,187	(−31,0)	<0,01
II	6	6,084±0,130	(−15,0)	<0,001	7,062±0,429	(−19,5)	<0,001
III	6	6,202±0,234	(+0,7)	>0,5	8,217±0,132	(−6,4)	<0,01
IV	6	7,800±0,234	(+9,0)	>0,05	8,327±0,077	(−5,0)	<0,001
V	6	6,916±0,0336	(−10,0)	>0,5	8,162±0,352	(−7,2)	>0,005
VI	6	8,372±0,364	(+17,0)	<0,05	7,865±0,143	(−10,4)	<0,001
IX	6	7,358±0,286	(+2,9)	>0,5	8,008±0,176	(−8,7)	<0,001
Hyperlipidemic animals receiving miscleron	10	6,188±0,234	(−13,0)	<0,01	6,842±0,044	(−22,0)	<0,001

Note. Here and in Table 3, the percentage changes in the factors in comparison with the controls are shown in brackets.

TABLE 3. Cholagogic Activity of Compounds Obtained (M ± m)

Test conditions		n	Amount of bile excreted over 3 h (ml)	Bile acid content of the bile, mg/ 100 ml	Cholesterol content of bile, mg/100 ml	Cholate–cholesterol coefficient
Untreated animals		11	1,7±0,1	532,0±47,4	34,3±2,9	14,2±2,0
Animals receiving:						
I		9	3,1±0,2 (+82,0)	443,0±27,0 (−17,0)	23,6±5,0 (−31,0)	20,5±2,7 (+44,0)
	p		<0,001	<0,5	<0,01	>0,05
II		5	2,0±0,2 (+17,0)	274,0±34,0 (−49,0)	18,9±2,3 (−45,0)	14,2±1,3 (0)
	p		<0,5	<0,01	<0,01	0
III		3	1,4±0,4 (−18,0)	304,0±21,6 (−43,0)	29,8±1,8 (−13,0)	10,4±1,3 (−36,0)
	p		<0,001	<0,01	<0,5	<0,5
IV		3	2,0±0,2 (+17,0)	250,0±20,0 (−53,0)	14,7±1,0 (−57,0)	17,4±2,4 (+22,0)
	p		<0,5	<0,01	<0,01	<0,5
V		4	1,8±0,2 (+6,0)	138,0±60,0 (−74,0)	10,4±2,7 (−70,0)	13,9±3,4 (−3,0)
	p		>0,5	<0,01	<0,01	>0,5
VI		6	1,8±0,4 (+6,0)	313,0±43,0 (−41,0)	13,5±1,6 (−60,0)	23,3±5,0 (+44,0)
	p		>0,5	<0,01	<0,01	<0,05
VII		4	1,2±0,1 (−41,0)	77,5±2,5 (−85,4)	29,0±0,1 (−15,0)	2,7±0,2 (−80,0)
	p		<0,01	<0,01	>0,5	<0,01
VIII		4	2,1±0,08 (+23,0)	138,7±25,0 (−74,0)	17,6±0,25 (−48,7)	7,8±1,2 (−45,1)
	p		<0,01	<0,01	<0,01	<0,001
IX		4	2,0±0,06 (+17,0)	285,0±1,5 (−47,0)	22,0±4,1 (−36,0)	13,7±2,0 (−4,0)
	p		<0,05	<0,01	<0,01	>0,5

The greatest chloagogic activity was also found in (I), this compound being more active than the cholagogic drug flamine. Unlike flamine, however, this compound had a much higher bile cholate–cholesterol coefficient, indicative of the antilithogenic properties of (I). The acute toxicity (LD_{50}) of this compound was 2000 mg/kg.

The α-cholest-5-enylcinnamic acids in general showed hypotriglyceridemic activity, with the exception of (V). Hypocholesteremic activity was seen only in (II). These compounds were also less active as cholagogs than (I). However, the cholagogic activity of (II), (IV), and (VIII) was equal to that of flamine. Compound (VI), which has a higher cholate–cholesterol coefficient, showed no appreciable effect on bile excretion. In contrast to the remaining compounds, (VII) markedly retarded the excretion of bile, and had a low cholate–cholesterol index, probably as a result of the presence of anitro-group in the aromatic ring.

These experimental findings thus confirm the hypothesis that introduction of carboxy- or carboxyalkyl-groups into the molecule enhances hypocholestermic and cholagogic activity.

EXPERIMENTAL (CHEMISTRY)

3-Carboxymethoxycholest-5-ene (I). This was obtained by boiling for one hour a solution containing 4.1 g (0.01 mole) of sodium cholesterate and 1.41 g (0.015 mole) of chloroacetic acid in 50 ml of benzene. The solvent was removed, and the residue washed with water and crystallized from ethanol to give 3.35 g (75%) of product.

Compounds (II-IX). A mixture of 4.4 g (0.01 mole) of (I), 0.01 mole of the appropriate aldehyde, 0.015 mole of triethylamine, and 2 ml of acetic anhydride was boiled for 24 h. After cooling, the reaction mixture was treated with 2 ml of water, heated for 30 min, and acidified with hydrochloric acid to pH 4-5. The solid which separated was washed with water until neutral, and crystallized from ethanol. The yields of reaction products (II-IX) were 70-90%. The elemental analyses for these compounds were in agreement with the values calculated from their empirical formulae, shown in Table 1.

EXPERIMENTAL (BIOLOGY)

Hypolipidemic activity was determined in white rats of both sexes, weighing around 200 g, with experimental hyperlipidemia induced by intraperitoneal administration of Triton WR-1339 [18]. The test compounds were administered orally simultaneously with the Triton, in doses of 10 and 75 mg/kg in the case of (I), and in a dose of 10 mg/kg for (II-IX). The reference material sued was the standard hypocholesteremic drug misclerone, in the therapeutic dose of 25 mg/kg [14]. Total blood serum cholesterol [9] and triglycerides [10] were measured. Cholagogic activity was examined in white rats of both sexes weighing 250-300 g in an acute test [12]. The bile acid and cholesterol contents of the bile were measured in three-hour portions of bile [15], and the cholate–cholesterol index calculated. The test compounds, and the reference cholagog flamine, were given orally in a dose of 75 mg/kg. The test results were treated by variational statistics [2]. The test results are shown in Tables 2 and 3.

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