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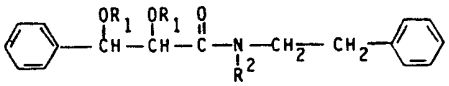
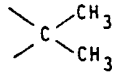
(54) α,β-dihydroxy benzene-ethanamides as natural products

(57) Compounds of the general formula



wherein
R_1 represents hydrogen, an acyl group having 1 to 18 carbon atoms or both R_1 together the group



R_2 represents hydrogen or a methyl group,
have been isolated in small amounts from the leaves of Clausena lansium.

The new compounds are to be used for the production of compositions for the use of the treatment of acute and chronical viral hepatitis, liver intoxication, hypoxia or amnesia.

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Formulae in the printed specification were reproduced from drawings submitted after the date of filing, in accordance with Rule 20(14) of the Patents Rules 1982.

SPECIFICATION

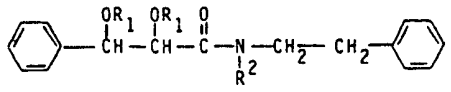
Novel amides, pharmacologically active compositions thereof, processes for the preparation thereof and their medical use

The present invention relates to new amides, their isolation from plants of the Rutaceae Clausena species, certain derivatives thereof and their use as hypoxiaprotective and antiamnestic agents. The invention is also concerned with pharmaceutical compositions containing said amides or its derivatives and with their manufacture.

Rutaceae Clausena anisata was reported to be used as a folk medicine in certain parts of Africa (I. Mester et al., Planta Medica 32 (1) 81, 1977). It has also been reported that the crude extract of Clausena indica Oliv. has cardiovascular activity and that two coumarin derivatives, Clausmarins A and B, isolated from Clausena pentaphylla (Roxb.) DC showed spasmolytic activity in animal test (Dhan Praktash et al., Phytochem. 17, 1194, 1978; Aboo Shoeb et al., J.C.S. Chem. Commun. 281, 1978). About fifty constituents have already been isolated from the roots, stems, etc. of various species of Clausena. Most of these constituents are derivatives of coumarin, carbazole and terpene; so far only two linear carboxylic acid amides were reported to be present in the leaves of Clausena plants (S.R. Johns et al., Aust. J. Chem. 20, 2795, 1967; Dhan Prakash et al., Indian J. Chem. Sect. B 19B (12), 1075, 1980).

It has now been found that the leaves of Clausena lansium contain new amides

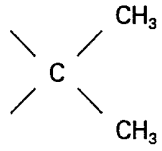
The present invention is directed to compounds of the general formula (I):



(I)

wherein

R_1 represents hydrogen, an acyl group having 1–18 carbon atoms or both radicals R_1 together the group



R_2 represents hydrogen or a methyl group

Preferred compounds according to general formula (I) are those wherein R_1 represents hydrogen or an acyl group having 1 to 4 carbon atoms and R_2 represents hydrogen or methyl.

Especially preferred are those compounds wherein R_1 represents hydrogen or the acetyl group.

The compound I, R_1=H, R_2=CH_3 apparently is a mixture of isomers arising from the differences in the relative position of the two hydroxyl groups (cis or trans). The present invention is also directed to the isolation of the compound I, R_1=H, R_2=CH_3 which comprises the steps of:

a) treating leaves of Clausena lansium with boiling water,

b) adding dilute acid (e.g. HCl) to the concentrated aqueous extract,

c) passing the supernatant through a column of cation ion exchange resin, preferably in its H^+-form,

d) treating the resin with a base, preferably aqueous ammonia,

e) extracting the resin with an organic solvent such as ethers, chloroform, methylene chloride, acetic acid esters of C_1–C_6 alcohols or C_2–C_6 ketones, preferably with diethyl ether,

f) chromatographing the concentrated extract on silica or aluminium oxide column with chloroform, methylene chloride, ether or chloroform/methanol mixture as eluting agent and

g) collecting and concentrating the eluate with an Rf-value of 0,37 on TLC (silica gel plate, $CHCl_3$:MeOH=91.3 as eluting agent).

It is preferred to recrystallize the crude products obtained by the above isolation methods from alcohols, e.g. methanol or ethanol.

Derivatives of the compound according to the general formula (I), wherein R_1 represents hydrogen may be synthesized by acylation and ketalization methods known per se.

The present invention also relates to pharmaceutical compositions and medicaments containing compounds of formula (I) as an active ingredient and to the manufacture of these compositions.

The invention is also directed to the use of compounds of formula (I) as hepatoprotective agents against chemical toxins and for increasing the detoxification function of the liver.

The acute toxicity of the compoundds of the formula (I) was found to be very low.

When tested for hepato-protection action the compounds according to the invention decreased

the elevated serum transaminase (SGPT) of mice intoxicated with CCl_4.

The pharmaceutical compositions according to the invention may for example take the form of ointments, gels, pastes, creams, sprays (including aerosols), lotions, suspensions, solutions and emulsions of the active ingredient in aqueous or non-aqueous diluents, syrups, granules or powders.

The compositions are preferably in the form of a sterile isotonic aqueous solution or in the form of tablets, capsules, pills and suppositories comprising a compound of the invention either alone or in admixture with a diluent.

The diluents to be used in pharmaceutical compositions (e.g. granules) adapted to be formed into tablets, dragees, capsules and pills include the following:

a) fillers, e.g. starch, sugars, and silicic acid; binding agents, e.g. cellulose derivatives, alginates, gelatine and polyvinyl pyrrolidone;

c) moisturizing agents, e.g. glycerol;

d) disintegrating agents, e.g. agar-agar, calcium carbonate and sodium bicarbonate;

e) resorption accelerators, e.g. quarternary ammonium compounds;

f) surface active agents, e.g. cetyl alcohol;

g) adsorptive carriers, e.g. kaolin and bentonite;

h) lubricants, e.g. talc, calcium and magnesium stearate and solid polyethylene glycols.

The tablets, dragees, capsules and pills formed from the pharmaceutical compositions of the invention can have the customary coatings, envelopes and protective matrices, which may contain opacifiers. They can be so constituted that they release the active ingredient only or preferably in a particular part of the intestinal tract, possibly over a period of time. The coatings, envelopes and protective matrices may be made, for example, of polymeric substances or waxes.

The ingredient can also be made up in micro-encapsulated form together with one or several of the above-mentioned diluents.

The production of the above-mentioned pharmaceutical compositions and medicaments is carried out by any method known in the art, for example by mixing the active ingredient(s) with the diluent(s) to form a pharmaceutical composition (e.g. a granulate) and then forming the composition into the medicament (e.g. tablets).

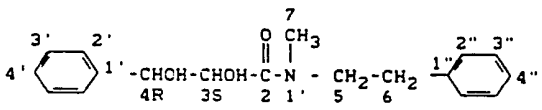
The pharmaceutical compositions according to the invention preferably contain about 0.1 to 99.5, more preferably from about 0.5 to 95% of the active ingredient by weight of the total composition.

The preferred daily dose for administration of the medicaments of the invention is 0.001 mg to 0.2 mg of the active ingredient.

The following Examples illustrate the invention.

Example 1

Isolation of 2,3-dihydroxy-N-methyl-3-phenyl-N-(2-phenylethyl)-propionic acid amide of the formula



80 kg of dried leaves of Clausena lansium (Lour) Skeels were boiled with water. The aqueous extract was concentrated to give 18 kg of crude syrup. 16 kg of the crude syrup were treated with 0.06 N HCl (80 l) and the supernatant was passed through a column of wet H± form cation ion exchange resin (from 48 kg of Na± form cation ion exchange resin). The resin was then washed with deionized water, treated with 2% aqueous NH_4OH (32.2 l) and finally extracted with diethyl ether (60 l) and finally extracted with diethyl ether (60 l). The concentrated ether extract as a syrup was chromatographed repeatedly on silica gel colums containing 20–100 times of its weight with chloroform as eluting agent. The conc. chloroform eluate with R_f=0.37 on the tlc was collected and concentrated. The crystals thus obtained were recrystallized from methanol. 11.18 g of white needles, m.p. 145 to 147°C were obtained.

$[\alpha]_D^{22}$ —65.8°C (0.19 in $CHCl_3$)

Elementary analysis:

	calculated for $C_{18}H_{21}NO_3$	found
C%	72.24	71.94
H%	7.08	7.10
N%	4.68	4.46

High resolution—MS M^+=299.1483

IR ν_{max}^{KBr} cm^{-1}: 3280 (OH), 3080, 3060, 3020, 1580, 1495, 750, 690 (monosubstituted benzene), 1620 (amide carbonyl)

UV λ_{max}^{MeOH} nm (logε): 256 (2.80)

H^1—NMR (in $CDCl_3$); chemical shift and assignment:

ppm (J, Hz)	hydrogen
2.70+2.90 (s; s; 3H)	N–CH_3
2.78 (t; J=6; 2H)	C_6H
3.42 (s; 2H, disappeared on addition of D_2O)	
3.51 (M; 2H)	C_5–H
4.35+4.56 (d; d; 1H; J=5)	C_3H or C_4–H
4.85 (d; 1H, J=5)	C_3H or C_4–H)
7.00–7.40 (m; 10 H)	aromatic H

^{13}C—NMR in $CDCl_3$; chemical shift and assignment:

carbon	ppm
2	173.5
3	76.3
4	73.2
5	51.1+52.0
7	34.3+35.2
6	36.0+36.5
1′	144.3
1″	140.0+140.3
the rest ten aromatic carbons	127.1 | 130.3

The compound reacted with HIO_4 to give benzaldehyde which was identified by its 2,4-dinitrophenylhydrazone.

Acetylation.

The compound was acetylated with acetic anhydride in pyridine to give an amorphous acetate.

Elementary analysis

	Calculated for $C_{22}H_{25}NO_{35}$	found
C%	68.93	68.69
H%	6.53	6.40
N%	3.66	3.95

IR, ν_{max}^{film} cm^{-1}: 1740(ester C=O), 1655(amide C=O, 1210(C–O)

MS, m/z(%): 383(M^+, 5), 292(M^+−91, 18), 249(292–43, 31), 207(249–43, 25).

^{1}HNMR in $CDCl_3$ chemical shift and assignment

δ ppm (J, Hz)	H
1.93 1.95 (two s 3H)	CH_3COO-
2.03 (s, 3H)	CH_3,COO–
2.95 3.06 (two s, 3H)	CH_3N-
2.84 3.01 (two t, J=7, 2H)	C_6–H
3.63 3.71 (two t, J=7, 2H)	C_5H
5.62 5.72 (two d, J=8, 1H	C_3–H
6.07 (d, J=8, 1H)	C4–H
7.2–7.5 (m, 10H)	aromatic H

Ketalization

The compound I, R=H was treated with acetone using hyperchloric acid as catalyst in room temperature to give crystals.

IR, $\gamma_{max}^{film}cm^{-1}$: 1665(amide C=O).

MS, m/z(5): 339(M^+, 5), 281(10), 190(28), 177(30), 162(32), 119(100), 105(74), 91(64), 77(25).

^{1}HNMR in $CDCl_3$ chemical shift and assignment

δ ppm (J, Hz)	H
1.46 1.52 (two s, 3H)	CH_3–C
1.80 1.85 (two s, 3H)	CH_3–C
2.31 2.65 (two m, 2H)	C_6–H
2.51 (s, 3H)	CH_3–N
3.15 (m, 2H)	C_5–H
4.99 5.20 (two d, J=7, 1H)	C_3–H
5.36 5.43 (two d, J=7, 1H)	C_4-H
7.00 | (m, 10H) 7.52	aromatic H

Example 2

Influence of the compound of the general formula I, R_1=H, R_2=CH_3, on liver functions.

Male Kunming strain mice weighing 18–22 grams were used throughout the experiments. The compound to be tested was suspended in 5% Tween 80 and given orally by gavage. The vehicle of 5% Tween 80 solution was administered to control mice via the same route. In in vitro experiments, the compound was dissolved in dimethylformamide and added directly into the incubation mixture.

The parameters adopted for hepatotoxicicity included serum tranaminase (SGPT), liver triglycerides, and pathological examination of liver tissues. The liver lesions were scored mainly by the extent of inflammation and necrosis and graded from 0 to 4.

1. Hepato-protective action.

1.1. Effect of the compound isolated from LCL on SGPT level of CCL_4-intoxicated mice.

Mice were divided into several groups.

The control group received the vehicle. The other groups were given two doses of the compound (250 mg/kg) at an interval of 8 h, respectively. All the mice were injected ip 10 ml/kg of 0.1% CCl_4 in vegetable oil 24 h after the last administration of the compound. The

mice were fasted for 16 h and sacrificed by decapitation. SGPT and liver lipids were determined. A piece of liver was processed into sectins for pathological examination.

As shown in table 1, the compound significantly decreased the elevated SGPT of mice intoxicated with CCl_4. 1.2 Protective action of the compound against CCl_4, acetaminophen and thiocetamide in mice.

In the experiments of anti-CCl_4 hepatotoxicity, mice were first injected 10 ml/kg of 0.15% CCl_4 in vegetable oil every other day for three doses. The treatment of mice with the compound (250 mg/kg daily) started from the second to fifth day after the first injection of CCl_4. The determination of SGPT and pathological examination of liver tissues were performed on day 7 of the experiment. The results indicate that the compound exhibited significant SGPT lowering action but had no effect on liver lesions (Table 2).

In another experiment, mice were first administered two doses of the compound and injected ip 10 ml/kg of 0.1% CCl_4 in vegetable oil 24 h after administration of the second dose. As shown in table 3, 250 mg/kg of the compound was effective for decreasing SGPT level and liver injuries, while 125 mg/kg was without effect.

In the experiment of thioacetamide hepatotoxicity, mice were treated according to the procedure of CCL_4 intoxication as described above except that thioacetamide 50 mg/kg was injected instead of CCl_4. The results showed that the compound markedly lowered the SGPT levels (table 4).

In the anti-acetaminophen hepatotoxicity experiment, mice received two doses (250 mg/kg) of the compound on the first day and the same dose on the second day. A dose of 150 mg/kg of acetaminophen was injected ip 6 h after the last dose of the compound. SGPT was determined and liver tissues were examined 20 h after acetaminophen injection. The compound was shown to be inactive.

1.3 Effect of the compound on serum and liver transaminase (GPT) levels of normal mice.

Groups of mice were administered the vehicle or 250 mg/kg of the compound once daily for 7 consecutive days. The serum and liver GPT were determined 24 h after the last dose administration. As shown in table 5, SGPT level of the mice treated with compound was slightly higher than that of the control but the difference was not significant. Similar results were obtained for liver GPT.

2. Mechanism of the protective action of the compound against CCl_4 hepatotoxicity.

CCl_4 hepatotoxicity has been considered to be due to formation of free radicals which initiate lipid peroxidation or covalent binding to macromolecules of microsomes. Thus, the structures and function of cell membranes were destroyed. The effect of the compound on CCl_4 induced lipid peroxidation (malonic dialdehyde, MDA, in vitro, and diene conjugates in vivo) and $^{14}CCl_4$ covalent binding to lipids and proteins of microsomes were investigated.

Lipid peroxidation:

MDA formation: The compound was pre-incubated with NADPH-reduced liver microsomes from phenobarbital-induced mice at 37°C for 15 minutes, 10 ul of CCl_4 (diluted with 20 volume of ethanol) was added into the incubation mixture, reincubated for 20 minutes. The production of MDA was determined by the method of thiobarbituric acid. The results indicate that 1 mM of the compound inhibited MDA production initiated by CCl_4 in vitro.

Diene conjugates: Mice were given two doses of 250 mg/kg of the compound on the first day and followed by the same dose on the second day and then received an injection of 1 ml/kg of CCl_4. The mice were killed 1 h after the CCl_4 injection. The diene conjugates in liver microsomes were extracted with chloroform-methanol (2:1) and washed with methanol 0.9% NaCl solution. The absorption of diene conjugates in the chloroform layer at 243 nm was measured with spectrophotometer. It was found that the compound could not inhibit diene conjugates production in livers from CCl_4-intoxicated mice (table 6).

$^{14}CCl_4$ covalent binding:

The condition of incubation was similar to that of MDA production. The compound was pre-incubated with MADPH-reduced liver microsomes for 10 minutes. The final concentration of the compound was 1 mM. $^{14}CCl_4$ 0.5 uci was added to the incubation mixture. Incubation was continued for another 60 minutes. The radio-activity of $^{14}CCl_4$ in microsomal lipids and proteins was separated by the method of extraction of diene conjugates and counted by liquid scintillation.

The results indicated that the covalent binding of $^{14}CCl_4$ to microsomal lipids and prteins was not inhibited by the compound.

3. Induction of hepatic microsomal cytochrome P-450.

The liver microsomal cytochrome P-450 plays a key role in the detoxification of xenobiotics. Since the compound was shown to be able to decrease hepatotoxicity induced by chemicals in

mice, it is of interest to study the effect of the compound on cytochrome P-450. Mice were administered 250 mg/kg of the compound once daily for 3 days. The control mice received the vehicle. The mice were killed after fasting overnight. Liver microsomes were prepared and microsomal monooxygenases were determined.

The data are shown in table 7, the hepatic cytochrome P-450, cytochrome b_5, NADPH-cytochrome c reductase, aminopyrine demethylase and benzo(a)pyrene hydroxylase activities were all increased significantly by the compound. The increase of liver size and microsomal protein content were not seen in the compound treated mice.

In another experiment, mice were given a dose of 250 mg/kg of the compound. Sodium pentobarbital (50 mg/kg) was injected ip 1 and 24 h after administration of the compound. The sleeping time was estimated by recording the interval of disappearance and recovery of righting reflex. The data are listed in table 8. The sleeping time of mice was shortened significantly by administration of the compound, 24 h before the injection of pentobarbital, the sleeping time of mice was prolonged markedly instead of being shortened when the compound was given 1 h prior to pentobarbital injection. However, prior adminstration of the compound did not affect sleeping time of mice injected with barbital which is not metabolized by the liver. It means that the prolongation of pentobarbital sleeping time by the compound was due to the inhibition of liver drug metabolism enzyme. Therefore, the compound has biphasic actions on the hepatic microsomal cytochrome p-450, i.e. inhibition followed by induction.

In conclusion, the compound of the general formula (I), R_1=H, R_2=CH_3 has hepato-protective action against CCl_4. The results of further investigations on the compound indicated that it also protected against thioacetamide hepatotoxicity, and that it could inhibit lipid peroxidation of microsomes induced by CCl_4. In addition, the compound has inducing action on hepatic cytochrome p-450.

Table 1. Effect of the compound of the general formula (I), R_1=H, R_2=CH_3 (250 mg/kg×2) isolated from the leaves of Clausena lansium (Lour) Skeels on SGPT levels of CCl_4 intoxicated mice (9 per group)

Constituents	SGPT unit % X±SE	P
Control	1678±261	
Compound (250 mg/kg×2)	727±266	<0.05

Table 2. Therapeutic effect of the compound on CCl_4 (0.15% in vegetable oil 10 ml/kg/day×3, PO) hepatotoxicity in mice (8 per group).

Group	SGPT unit % x±SE	P
Control	2695±110	
Compound (250 mg/kg/day×4)	1801±312	<0.01

Table 3. Protective action of the compound against CCl_4 (0.1% 10 ml/kg, i.p) hepatotoxicity in mice

	Control	Compound 125 mg/kg	250 mg/kg
SGPT unit %	3016±23	2727±162	2270±221
Liver lipids mg/g	21.0±4.2	21.0±3.8	22.5±5.5
Liver lesions			
Inflammation (Grade)	1.70	—	0.16
Necrosis (Grade)	2.33	—	0.72

Nine mice per group. ±SE.
<0.01

Table 4. Protective action of the compound against thioacetamide (50 mg/kg, ip) hepatotoxicity in mice

Group	SGPT unit % ±SE	Liver lipids mg/g±SE
Control	1696±231	61±11.7
Compound (250 mg/kg×2)	1045±166	59±11.3

Nine per group.
<0.05.

Table 5. Effect of the compound on serum and liver transaminase (GPT) level of normal mice (8 per group)

Group	SGPT unit %±SE	LGPT unit %±SE
Control	217±17.5	260±11.6
Compound (250 mg/kg/day×7)	272±27,8	234±13.6

Table 6. Effect of the compound on CCl_4-initiated lipid peroxidation of liver microsomes (MDA) in vitro and diene conjugates in vivo)

	Control	Compound
MDA OD×100/1.5 mg	35.5±(3)	29.7±4(3)
Inhibition %		16.3
Diene conjugates OD×100/g liver	55.8±2.3(8)	54.8±3.3(8)
Inhibitation %		

Figures in the parenthesis are number of the determinations.

Table 7. Induction of hepatic microsomal cytochrome P-450 by the compound in mice (6 per group)

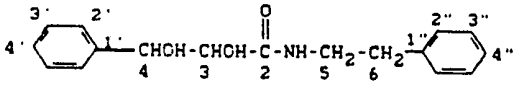
	Control	Compound 250 mg/kg day x 3	P
Liver weight g %	4.0±0.2	4.3±0.2	> 0.05
Microsomal protein mg/g liver	6.3±0.3	7.0±0.5	> 0.05
Cytochrome P-450 nmol/mgprotein	0.87±0.07	1.23±0.06	< 0.01
NADPH-cytochrome C reductase, nmol cytochrome c reduced/min/mg protein	103±2.5	148±2.5	< 0.01
Cytochrome b_5 nmol/mg protein	0.15±0.01	0.16±0.016	> 0.05
Aminopyrine demethylase, nmol HCHO/min/mg protein	81±63	107±5.8	< 0.01
A H H nmol/min/mg protein	2.6±0.4	6.2±0.42	< 0.01

Table 8. Effect of the compound on barbiturates sleeping time in mice (10 per group)

Barbiturate	Group	Interval between compound and barbiturate	Sleeping time X ± SE	P
Pentobarbital 50 mg/kg	Control		71±7	
	Compound	1 h	132±13	<0.01
	Compound	24 h	45±6	<0.01
Barbital 200 mg/kg	Control		197±27	
	Compound	1 h	172±13	>0.05

EXAMPLE 3

Isolation of the compound of the formula (so-called compound 7)



80 kg of dried leaves of Clausena lansium (Lour) Sdeels were boiled with water. The aqueous extract was concentrated to give 18 kg of crude syrup. 16 kg of the crude syrup were treated with 0.06 N HCl (80 l) and the supernatant was passed through a column of wet H^+—form cation ion exchange resin (from 48 kg of Na^+—form cation ion exchange resin). The resin was then washed with deionized water, treated with 2% aqueous NH_4OH (32.2 l) and finally extracted with diethyl ether (60 l). The concentrated ether extract was treated repeatedly on silica gel columns (ratio varied from 100:1 to 2:1) with chloroform as eluting agent. The concentrated chloroform eluate with Rf=0.28 on tlc was collected and concentrated. The crystals thus obtained were recrystallized from methanol. 0.3 g of white prism crystals, m.p. 151–152°C were obtained $[\alpha]_D^{28}$+38° (0.30 in MeOH).

Elementary analysis:

Calculated for $C_{17}H_{19}NO_3$	found	
C%	71.58	71.86
H%	6.67	6.47
N%	4.91	4.68

High resolution —MS M^+=285.1395

IR γ_{max}^{KBr} cm^{-1}: 3300 (OH), 3190, 1570(N–H), 1655(amid C=O) 3060, 3030, 1605, 1495, 760, 700 (monosubstituted benzene).

UV λ_{max}^{MeOH} nm (logε): 255 (2.60)

MS m/z(%): 285(M^+, 1), 267($M-H_2O$, 0.3), 179(45), 148(6), 120(6), 105(100), 91(48), 77(64).

^{1}H-NMR (in $CDCl_3$); chemical shift and assignment;

ppm (J, Hz)	hydrogen
2.95(m, 2H)	C_6–H
3.25(m, 2H)	C_5–H
4.05(t, J=4, 1H)	C_3–H
4.82(t, J=4, 1H)	C_4–H
5.45(d, J=4, 1H, disappeared on addition of D_2O)	OH
5.55(d, J=4, 1H, disappeared on addition of D_2O)	OH
7.02–7.39(m, 10H)	aromatic H
7.44 (br, 1H)	N–H

^{13}C–NMR in $CDCl_3$; chemical shift and assignment:

carbon	ppm
2	171.3
3	73.7
4	73.7
5	38.9
6	34.4
1′	137.6
1″	139.7
aromatic carbon	125.1–127.4

Derivative of compound of Example 3 (so-called compound 7)

(i) Compound 7i

Compound 7 was acetylated with acetic anhydride to give white syrup. The spectrometric data indicated the compound is the acetate of compound 7.

IR γ_{max}^{film} cm^{-1}: 3340(N–H), 1760, 1235(ester C=O), 1680(amide C=O), 3080, 3040, 1610, 1500 (monosubstituted benzene)

MS m/z(%): 369(M^+, 0.5), 309(M-60, 0.8), 267(309–42, 3) 249(309–60 or 267–H_2O, 20), 250(249–1, 53), 179(20), 148(18), 120(30), 105(35), 104(40), 91(38), 77(18), 43(100).

^{1}HNMR in $CDCl_3$; chemical shift and assignment:

δ	H
2.02(s, 3H)	CH_3COO
2.05(s, 3H)	CH_3COO
2.59(m, 2H)	C_6–H
3.39(m, 2H)	C_5–H
5.65(d, J=4, 1H)	C_3–H
5.88(br, 1H)	N–H
6.30(d, J=4, 1H)	C_4–H
6.89–7.08(m, 2H)	aromatic H
7.15–7.50(m, 8H)	

(ii) Compound 7ii

Compound 7 was treated with acetone using hyperchloric acid as catalyst in room temperature to give transparent syrup compound 7ii.

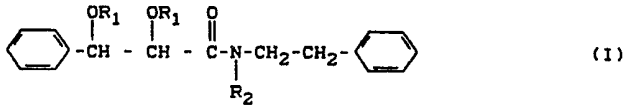
MS m/z(%): 325(M^+, 10), 267(40), 177(15), 148(14), 120(100), 119(80), 105(55), 91(75), 77(30).

^{H}NMR in $CDCl_3$; chemical shift and assignment;

δ	H
1.50 (s, 3H)	CH_3–C
1.65 (s, 3H)	CH_3–C
2.49 (t, J=7, 2H)	C_6–H
3.20 (m, 2H)	C_5–H
4.85 (d, J=7, 1H)	C_3–H
5.48 (d, J=7, 1H)	C_4–H
6.35 (br, 1H)	N–H
7.08–7.50 (m, 10H)	aromatic H

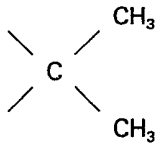
CLAIMS

1. Compounds of the general formula (I)



(I)

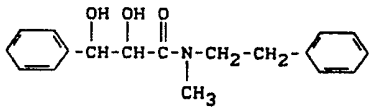
wherein R_1 represents hydrogen, an acyl group having 1–18 carbon atoms or both radicals R_1 together the group



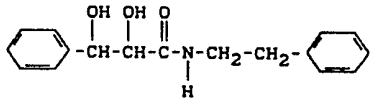
and R_2 represents hydrogen or a methyl group.

2. Compounds of the general formula (I) in claim 1, wherein R_1 represents hydroge or an acyl group having 1–4 carbon atoms and R_2 represents hydrogen or a methyl group.

3. The compound of the formula



4. The compound of the formula



5. A method for the isolation of the compound of the general formula (I), R_1=H, R_2=H or CH_3, preferably CH_3, in claim 1, which comprises the steps of:

a) treating leaves of Clausena lansium with boiling water,

b) adding dilute acid (e.g. HCl) to the concentrated aqueous extract,

c) passing the supernatant through a column of cation ion exchange resin, preferably in its H^+-form,

d) treating the resin with a base, preferably aqueous amonia,

e) extracting the resin with an organic solvent such as ethers, chloroform, methylene chloride, acetic acid esters of C_1–C_6 alcohols or C_2–C_6 ketones, preferably with diethyl ether,

f) chromatographing the concentrated extract on silica or aluminium oxide column with chloroform, methylene chloride, ether or chloroform/methanol mixture as eluting agent and

g) collecting and concentrating the eluate with an Rf-value of 0,37 on TLC of silica gel plate, $CHCl_3$: MeOH=97:3 as eluting agent.

6. A pharmaceutical composition containing as an active ingredient a compound according to any of claims 1 to 4.

7. A process for the preparation of a pharmaceutical composition according to claim 6, characterized in that a compound according to any of claims 1 to 4 is mixed with a diluent and/or other additives or auxiliary agents.

8. A compound according to any of claims 1 to 4 for use in the treatment of diseases.
9. A compound according to any of claims 1 to 4 for use in the treatment of acute and chronical viral hepatitis, liver intoxication, hypoxia or amnesia.

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