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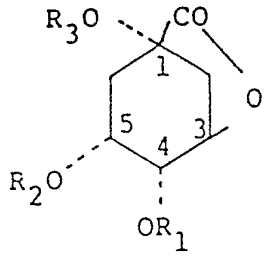

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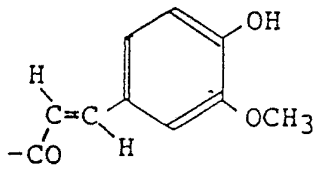

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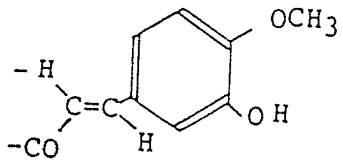

III

(57) Abstract

Compounds of general formula (I), wherein one of the R_1, R_2 and R_3 groups represents a feruloyl or isoferuloyl group of formula (II) or (III), respectively, and the other R_1, R_2 and R_3 groups represent hydrogen. These compounds have opiate receptor activity and may be used in treatment of the toxic effects of opiate narcotic analgesics. Preparation of these compounds by extraction from coffee, and by synthetic processes is disclosed.

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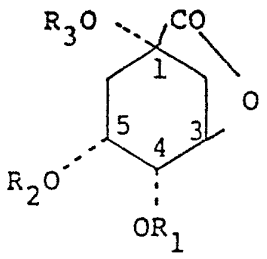
"OPIATE ANTAGONISTS"

This invention relates to the identification and isolation of compounds having opiate receptor binding activity, to the synthesis thereof, and to the use of such compounds as opiate narcotic antagonists.

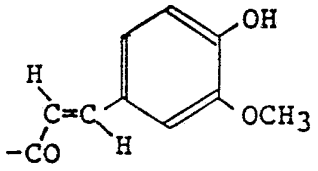
Previous reports have shown that solutions of instant coffee powders, both normal and decaffeinated, contain ligands for opiate receptors with characteristics similar to those of known opiate antagonists such as naloxone (Boublik, J.H. et al. Nature 301, 246-248 (1983)). Further work has now enabled the isolation of the principal component of this opiate receptor binding activity and established the identity thereof.

According to the present invention there are provided compounds having opiate receptor binding activity of the general formula I



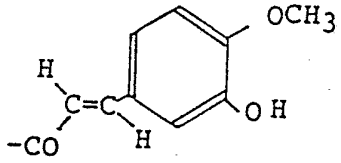
I

wherein one of the R_1, R_2 and R_3 groups represents a feruloyl or isoferuloyl group of the formula II or III, respectively:



II

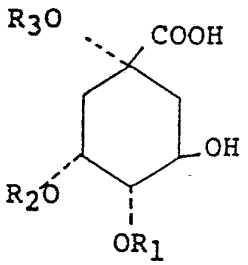
and



III

and the other R_1, R_2 and R_3 groups represent hydrogen.

Compounds of the general formula I above may be derived by pyrolysis from the corresponding feruloylquinic acids of the general formula IV

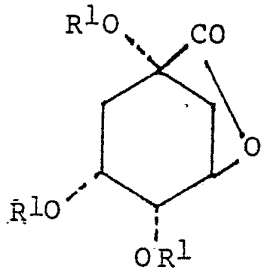


IV

wherein R_1, R_2 and R_3 are as defined above. Feruloylquinic acids of the general formula IV are known to occur in green coffee beans (Clifford, M.N., Food Chem. 4, 146-149 (1961)).

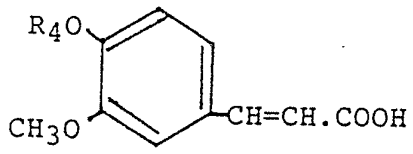
Compounds of the general formula I may also be isolated directly from coffee beans which have been roasted, ground and extracted with water and from instant coffee. This process, which includes thes teps of adsorption on an ion exchange resin followed by chromatography to isolate fractions having the desired activity, is described in greater detail below.

The present invention also provides a process for the preparation of the compounds of the general formula I, above, which process comprises the reaction of quinic- 1,3-lactone (or quinide) of the formula V:

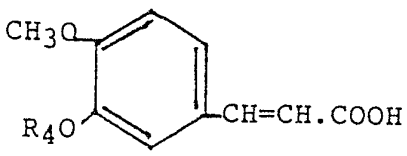


V

wherein each R^1 separately represents H or a removable hydroxy protecting group, with a ferulic or isoferulic acid of the formula VI or VII, respectively:



VI



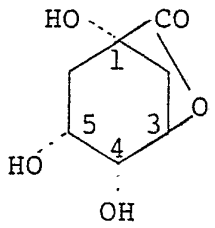
VII

wherein R_4 is an acetyl or other hydroxy protecting group, or a reactive derivative thereof, followed by removal of the group R_4 and, if necessary any hydroxy protecting groups R^1.

The lactone of the formula V may be prepared from quinic acid by reaction with a condensing agent, for example dicyclohexylcarbodiimide (DCC), or by other known methods, such as by reaction with gaseous HCl in acetone. Removable hydroxy protective groups R^1 may be added by methods known per se in the art.

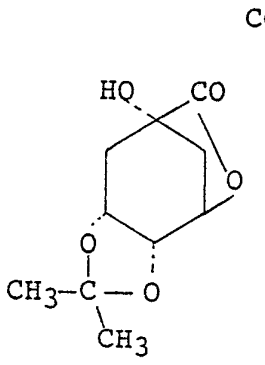
In one embodiment of the process of this invention, quinic acid is converted to quinic-1,3-lactone (V) in the presence of DCC, and then acetyl ferulic acid or acetyl isoferulic acid (VI, VII, R_4= CH_3CO-) is added. Following the formation of acetylferuloylquinides or acetylisoferuloylquinides, the acetyl group is removed by brief exposure to methanolic K_2CO_3.

Preferably, the process of the invention involves the reaction of quinic-1,3-lactone of the formula

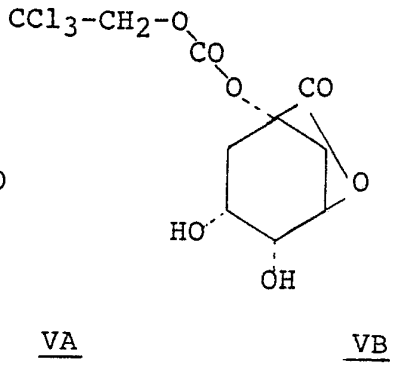


with the hydroxyls on carbons 4 and 5 blocked with an isopropylidene group (formula VA), or the hydroxyl on carbon 1 protected with a 2,2,2-trichloroethoxy carboxy

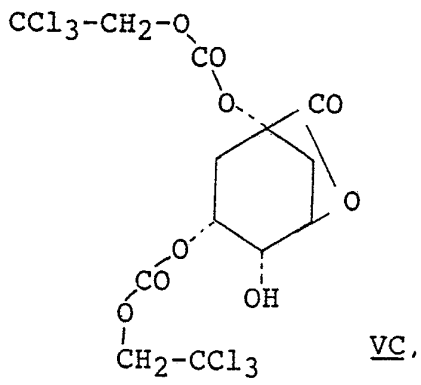
ester (formula VB) or both hydroxyls 1 and 5 similarly esterified (formula VC):



VA

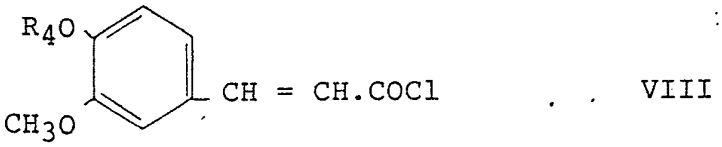


VB

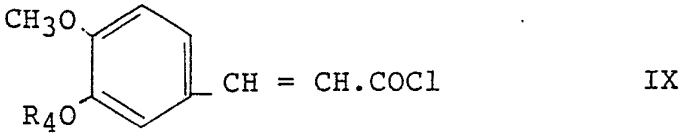


VC,

with a feruloyl or isoferuloyl chloride of formula VIII or IX respectively.



VIII



IX

wherein R_4 is an acetyl or other hydroxy protecting group, followed by removal of the group R_4. Similarly, the protecting isopropylidene or trichloroethoxy carbonyl groups are removed to give in the cases of VA, VB, and VC, the 1,5, and 4-substituted esters of quinic-1,3-lactone respectively.

Compounds of the general formula I exhibit significant opiate receptor binding activity, and thus have potential for use as opiate antagonists in

reversing the toxic effects of opiate narcotic analgesics such as morphine, for example in the treatment of opiate narcotic overdosage and in the reversal of respiratory depression following the use of narcotics during surgery.

The present invention therefore also extends to pharmaceutical compositions comprising a compound of the general formula I, together with one or more pharmaceutically acceptable carrier or diluent; as well as to methods of treatment of the conditions outlined above, which comprise administration to a patient of a compound of the general formula I, or a pharmaceutical composition containing a compound of the general formula I.

Preliminary attempts to isolate opiate receptor-active material in instant coffee by solvent extraction and chromatography indicated that activity was associated with yellow pigment having an absorption maximum of 325 nm, which is characteristic of the chlorogenic acids. On silica chromatography, the activity was less polar than the most nonpolar of the chlorogenic acids, i.e. the dicaffeoylquinic acids or "isochlorogenic acid" as isolated from green coffee beans (Sondheimer, E., Szymanski, C.D. & Corse, J.W. J. Agr. Food Chem. 9, 146-149 (1961)). Further, it was non-acidic, being not adsorbed by DEAP-LH20 anion exchanger (acetate-form) in 72% aq. ethanol (Setchell, K.D.R., Alme, B., Axelson, M. & Sjovall, J. J.steroid Biochem. 7, 615-629 (1976)). In common with many of the caffeoylquinic acids, it was prone to oxidation, particularly on silica during chromatography.

Isolation was monitored by an assessment of ED_{50} values in an opiate receptor assay described hereinafter in detail (see Table 1 below). Fig.1 details the chosen isolation scheme which resulted in a twenty-fold increase in specific activity, i.e. from an ED_{50} of 1.2 mg/ml for instant coffee to 0.05 mg/ml for the final extract. The GC-MS (gas chromatogram-mass spectra) findings are presented in Fig.2A. Principal total ion peaks of the gas chromatogram contained M/Z 249 (derivatized feruloyl ions) and five of these also exhibit the same molecular ion M/Z 566. The five, with similar mass spectra, are from the possible feruloylquinides of general formula I. Some may be cis-isomers produced as artifacts by the conditions during gas chromatography. Another five hidden peaks of derivatized caffeoyl ions M/Z 307, totalling 10% of the feruloyl ions were also observed and were assumed to represent demethylation that occurred during isolation.

It has been shown (Reichstein, T. Helv. chim. Acta 15, 1450-1453 (1932)) that a compound, subsequently called Hauschild's Substance, isolated from the beverage "mate" (Hauschild, W. Mitt. Levensmitt. Hyg. 26, 329 (1935) (Chem. Abs. 30, 3537 (1936)) was a lactone of neochlorogenic acid (5-caffeoylquinic acid) formed during the preparation of the drink from Ilex paraguariensis. In view of the fact that green coffee beans may contain up to 10%, on a dry weight basis, of chlorogenic acids, of which 75% are caffeoylquinic acids (Clifford, supra), it is highly probable that Hauschild's Substance, and other caffeoylquinides, would be present in roasted and instant coffee. To test whether these contribute to opiate receptor activity, pure chlorogenic acid, 3-caffeoylquinic acid, isolated

from green coffee beans (Sandheimer et al, supra) was heated to 90°C in saturated $NaHCO_3$ solution for 30 minutes. This is known to produce a mixture of 4- and 5-caffeoylquinic acids (Haslam, E., Makinson, G.K., Naumann, M.O. & Cunningham, J. J.chem. Soc. 2137-2146 (1964)). The mixed acids were then lactonized by heating to 90°C for 3 hours in glacial acetic acid. Flash chromatography by the same system in Fig.1 lead to a product with negligible opiate receptor activity (Table 1). The same product on GC-MS showed two peaks, presumably 4- and 5-caffeoylquinides (Fig.2B). Although these probably represent the usually more stable trans isomers and the cis forms still remain to be tested, this is in accordance with the observation (Table 1) that the 3-methyl ether of 3,4-dihydroxystyrene (4-vinylguaiacol) has appreciable opiate receptor binding activity while the unmethylated parent compound has none.

It has been noted that opiate receptor activity develops in coffee beans with roasting, and a comparison of the Arabica and Robusta species of coffee shows that the latter can produce a higher level of activity when each is identically roasted, ground, and extracted with hot water (Table 1). As it is a well documented observation that Robusta green beans contain about twice the level of feruloylquinic acids as Arabica, (Clifford, supra; Rees, D.I. & Theaker, P.D. 8th International Scientific Colloquium on Coffee, Abidjan, 1977, ASIC (Paris) 79-84 (1979)), this finding is compatible with the generation of feruloylquinides during roasting and the difference in opiate receptor activity of the two brewed coffees.

Robusta green beans can have a content of up to 2% feruloylquinic acids (Rees et al, supra), so if it is assumed that instant coffee has been manufactured from beans with a 1.5% content, then, in a "medium" roast (Rees et al, supra), about two-thirds of this could be dehydrated to feruloylquinides. Conversion of the roasted bean to instant coffee is estimated to double the concentration of active quinides - possibly to 2% of instant coffee. If this figure is applied to the ED_{50} for instant coffee set out in Table 1 (1.2 mg/ml) it would indicate that feruloylquinides have an ED_{50} of 0.024 mg/ml. The finding of 0.05 mg/ml for crude feruloylquinides indicates that one or more of the isomeric forms of feruloylquinide produce the predominant opiate receptor binding activity of instant coffee.

TABLE 1. The half-maximal effective dose (ED_{50})* of materials derived from, or known to occur in, roasted coffee.

	ED_{50} (mg/ml)
Brewed Arabica Coffees+	3.0, 3.9 (14.7 14.6)
Brewed Robusta Coffees+	2.1, 1.8 (7.6 10.8)
Instant Coffees (Different Types)	1.1 ± 0.1 (mean ± s.e.m.; n-43)
Product of treatment of instant coffee with Amberlite XAD-2 and C18 silica (Fig.1)	0.45
Flash chromatography fractions (Fig.1)	
No. 7	>10
8	0.35
9	0.25
10	0.1
11	0.05
12,13	0.1
14,15,16	0.15
17,18	0.2
19,20	0.6
4- and 5-caffeoylquinides (see text)	>10

3,4-dihydroxystyrene, synthesized (Rahn,W. & Konig, W.A. J. High Resolution Chromatogr. Commun.1, 69-71 (1978))	>10
4-vinylguaiacol,synthesized (Reichstein, T. Helv. Chim. Acta 15, 1450-1453(1932))	1.2

TABLE LEGEND

* ED_{50}'s represent the concentration of material required to displace by 50% the binding of ^{3}H-naloxone in a crude rat brain membrane preparation as described in detail hereinafter. Samples were dissolved in 0.05 M Tris (HCl buffer at pH 7.4 and assayed in triplicate at 3 to 5 concentrations. ED_{50} values are determined graphically on log transformed data.

\+ Two varieties each of Arabica and Robusta green beans were roasted to 15% weight loss. Each was ground to a particle size <0.5 mm and 10 g samples were extracted with 10 ml water at 100°C in a single pass expresso coffee maker. Each brew was lyophilized and dry residues weighed and redissolved for assay. ED_{50} values represent the concentrations with respect to the mass of dry residue. Due to different extraction percentages for the four varieties (ranging from 16-27%), the ED_{50} values in green bean mass equivalents are also presented (in brackets).

Further details of the isolation of opiate receptor-activity material in instant coffee powder, and of the characterisation thereof, and of the synthesis of the compounds of the invention, are set out in the following Examples:

EXAMPLE 1

A. Isolation Scheme (see Figure 1).

1. An aqueous suspension of instant coffee (2.5 g/100ml) was passed through a column of Amberlite XAD-2 (7 g/g coffee powder) and the column washed with an equal volume of water. Adsorbed material was eluted with 80% aqueous methanol and the methanol evaporated in vacuo. Then residue was adjusted to 20% aqueous methanol.

2. Solution was stirred with reverse phase C18 silica (from Waters SEP-PAK cartridges) 1 g/10 ml, for at least 10 minutes, filtered, and washed with 20% aqueous methanol until no further colour eluted. Then C18 silica extracted with 60% aqueous methanol (10 ml/g) and filtered solution initially evaporated in vacuo to an aqueous suspension, then, lyophilized to light brown flakes.

3. A column of silica (Merck Kieselgel 60, 230-400 mesh) was prepared for flash chromatography (Still, W.C., Kahn, M. & Mitra, A. J. Org. Chem. 43, 2923-2925 (1978)) with $CHCl_3$-CH_3OH (80:20 v/v) and coffee concentrate applied to the top as a suspension. Volume of developing solvent and size of fractions were as recommended (Still et al, supra) with positive pressure to give a solvent

drop of 1 cm/min. After removal of solvent, each fraction was assessed by an ED_{50} determination in the opiate receptor binding assay as detailed below. The results are shown in Table 1. Fractions with an ED_{50} of 0.1 mg/ml or less were combined.

4. Preparative gel permeation chromatography using Waters HLPC instrumentation (model 6000A pump, model UK6 injector, model 450 variable wavelength detector) and a Waters Ultrastyragel 100Å column, was carried out with tetrahydrofuran (THF) as solvent at 1 ml/min. Injections of extract in THF (10 mg/ml) was 100 µl and 0.2 ml fractions collected. Analytical assessment of each fraction was made with 3 µl injections and detection at 300 nm. Most of the UV absorbing material had a retention volume of 6.93 ml and those preparative fractions with a single peak at 6.93 ml, were combined and again reprocessed by preparative GPC as above. After the second purification, the combined fractions were evaporated with N_2 and dried prior to GC-MS.

Figure 2 shows mass chromatograms of (A) TMS derivatives of instant coffee extract (Fig.1) showing time profiles of total ion current, ions m/z 249 (feruloyl ion), and m/z $566^{(M+)}$ and (b) TMS derivatives of 4- and 5-caffeoylquinides showing time profiles of total ion current, ion m/z 307 (caffeoyl ion), and m/z $624^{(M+)}$. Derivatives were prepared by reaction of sample with 100 µl trimethylsilylimidazole (Pierce) and 100 µl dry acetonitrile for 30 min at 60°C in sealed vials. One microlitre was injected into gas

chromatograph with temperature program 200°C to 300°C at 10°C/min with final hold time of 10 min at 300°C. Carrier gas was helium at 30 ml/min and injection and detector zones were 300°C. The column (2 m x 2 mm ID) was packed with 3% SP2100 on chromosorb W. Detection of the effluent was by mass spectrometry (Finnigan Model 4021) using electron impact ionization at 70 eV and a source temperature of 250°C.

B. Opiate Receptor Binding Assay

The receptor assay system was based on that of Pert and Snyder (Pert, C.B. & Snyder, S.H., Proc. Natu. Acad. Sci. U.S.A. 70, 2243-2247, (1973)). Male Sprague-Dawley rats were killed by decapitation, and brains minus cerebellum homogenized in 25x vol of 0.05 Tris-HCl, pH 7.4 buffer using a Brinkmann Polytron on setting 4 for 5 s. The homogenate was centrifuged for 20 min at 18,000 g, and the pellet resuspended in 25x vol buffer. Aliquots (0.5 ml) of this preparation were incubated with 1 nM ^{3}H-naloxone (40 Ci mmol $^{-1}$; NEN) alone or with various test preparations. Incubations were done in 0.5% BSA and 10% Trasylol for 30 min at 25°C, and terminated by rapid vacuum filtration through Whatman GF/B glass fibre filters pre-wetted with buffer containing 0.5% BSA. Filters were washed with 5 ml buffer, air-dried and added to 10 ml non-aqueous scintillant.

EXAMPLE 2

a. Preparation of quinic-1,3-lactone

Quinic acid in anhydrous DMF (dimethylformamide) was added to DCC (dicyclohexylcarbodiimide) in DMF magnetically stirred at room temperature. Pyridine was

added and after stirring for 30 minutes, the solution went turbid. Stirring was continued for a further two and a half hours.

b. Preparation of acetylferulic acid

Acetylferulic acid was prepared after the method of L.S.Fosdick & A.C.Starke, J.Am.Chem.Soc. 62, 3352-5 (1940).

c. Preparation of acetylferuloylquinides

Quinic-1,3-lactone and acetylferulic acid prepared as described above were reacted to form the desired esters.

d. Preparation of Feruloylquinides

Acetylferuloylquinide from the previous preparation was dissolved in methanol and K_2CO_3 dissolved in aq.methanol was added.

EXAMPLE 3

Using acetylisoferulic acid (prepared previously by J.Pacsu and C.Stieber, Ber. 62B, 2974-9 (1929)), acetylisoferuloylquinides may be prepared exactly as described previously for acetylferuloylquinides. Removal of the acetyl group as described above yields the desired isoferuloylquinides.

EXAMPLE 4

Reaction of 4,5-O-isopropylidenequinide (prepared as described by H.O.L.Fischer, Chem.Ber. 54, 775-83 (1921)) with acetyl feruloyl chloride (prepared as described by L.S.Fosdick and A.C.Starke, supra), yields 1-O-acetylferuloyl-4,5-O-isopropylidenequinide. The

hydrolytic removal of the 4,5-O-isopropylidene group from the 1-O-substituted quinide may be performed by known methods (for example, as described by J.D.Elliott, M. Hetmanski, R.D.Stoodley, M.N. Palfreyman, J.C.S.Perkin I, 1782-88 (1981)). The acetyl group may be removed by the method described previously to yield the 1-feruloyl ester of quinic-1,3-lactone.

By analogous methods using acetylisoferuloyl chloride instead of acetylferuloyl chloride, the 1-isoferuloyl ester of quinic-1,3-lactone may be prepared.

EXAMPLE 5

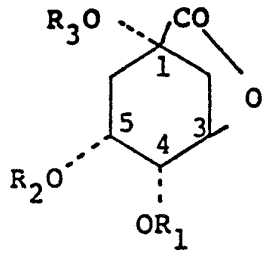
The general procedures of Example 4, may be repeated using 2,2,2-trichloroethoxycarboxy esters as protecting groups on the 1-, or 1- and 5-, hydroxy groups of quinic-1,3-lactone, following the methods for synthesis and facile hydrolysis described by T.B.Windolz and D.B.R.Johnston, Tetrahedron Lett. 2555 (1967).

EXAMPLE 6

Hydrolytic removal of 4,5-O-isopropylidene group from 1-O-acetylferuloyl-4,5-O-isopropylidenequinide (described as an intermediate in the synthesis of 1-feruloylquinic acid by A.Zane and S.H.Wender, Chem.& Ind. 1034-5 (1965)) by the method of Elliott et al; supra, followed by removal of the acetyl group by the method described previously yields the 1-feruloyl ester of quinic-1,3-lactone.

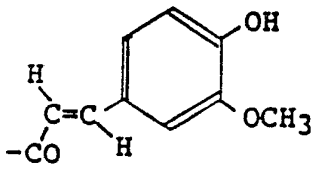
CLAIMS:

1. A compound having opiate receptor binding activity of the general formula I:



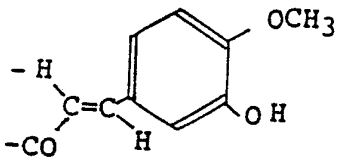
I

wherein one of the R_1, R_2 and R_3 groups represents a feruloyl or isoferuloyl group of the formula II or III, respectively:



II

and



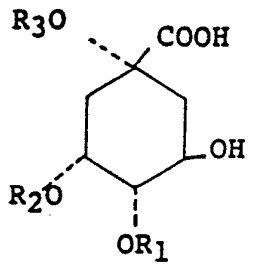
III

and the other R_1, R_2 and R_3 represent hydrogen.

2. A compound of the general formula I defined in claim 1, in substantially pure form.

3. A process for the preparation of a compound of the general formula I as defined in claim 1, which comprises extraction from instant coffee or from coffee beans which have been roasted, ground and extracted with water, said extraction including the steps of adsorption on an ion exchange resin, followed by chromatography to isolate fractions having the desired activity.

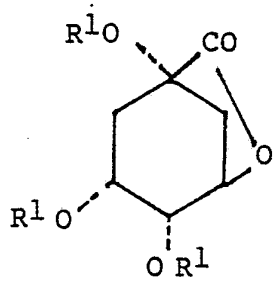
4. A process for the preparation of a compound of the general formula I as defined in claim 1, which comprises pyrolysis of a corresponding feruloylquinic acid of the general formula IV:



IV

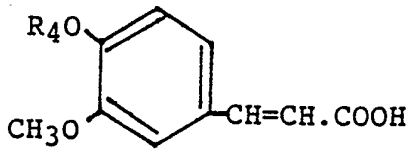
wherein R_1, R_2 and R_3 are as defined in claim 1.

5. A process for the preparation of a compound of the general formula I as defined in claim 1, which process comprises the reaction of quinic-1,3-lactone of the formula V:

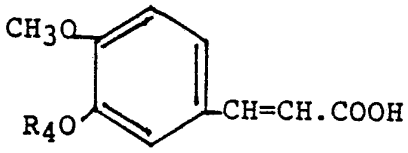


V

wherein each R_1 separately represents H or a removable hydroxy protecting group, with a ferulic or isoferulic acid of the formula VI or VII, respectively:



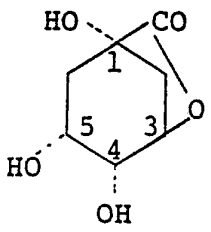
VI



VII

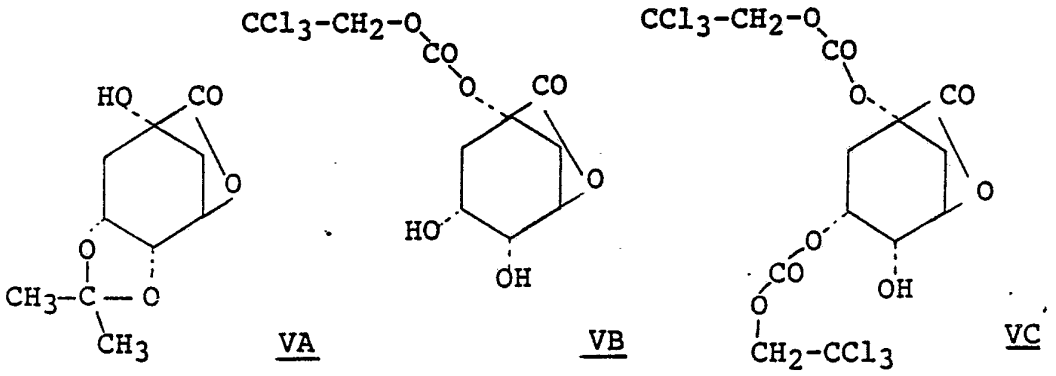
wherein R_4 is an acetyl or other hydroxy protecting group, or a reactive derivative thereof, followed by removal of the group R_4 and, if necessary, any hydroxy protecting group R^1.

6. A process as defined in claim 5, which comprises the reaction of quinic-1,3-lactone of the formula



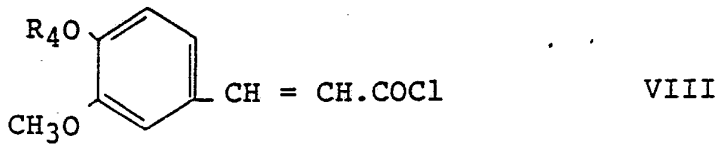
with the hydroxyls on carbons 4 and 5 blocked with an isopropylidene group (formula VA), or the hydroxyl on

carbon 1 protected with a 2,2,2-trichloroethoxy carboxy ester (formula VB) or both hydroxyls 1 and 5 similarly esterified (formula VC):

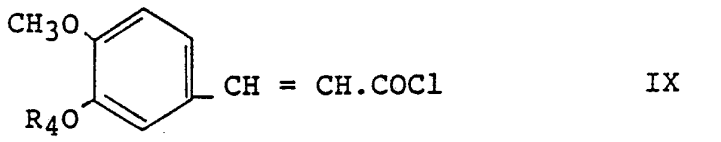


VA VB VC

with a feruloyl or isoferuloyl chloride of formula VIII or IX respectively:



VIII



IX

wherein R_4 is an acetyl or other hydroxy protecting group, followed by removal of the group R_4 and the protecting isopropylidene or 2,2,2-trichloroethoxy carbody groups.

7. A pharmaceutical composition for the treatment of the toxic effects of opiate narcotic analgesics, comprising a compound of the general formula I as defined in claim 1, together with one or more

pharmaceutically acceptable carriers or diluents therefor.

8. A method of treatment of the toxic effects of opiate narcotic analgesics, which method comprises administration of an effective amount of a compound of the general formula I as defined in claim 1, or of a pharmaceutical composition containing a said compound.

9. Use of a compound of the general formula I as defined in claim 1, for the treatment of the toxic effects of opiate narcotic analgesics, or for the manufacture of a pharmaceutical composition for the treatment of the toxic effects of opiate narcotic analgesics.

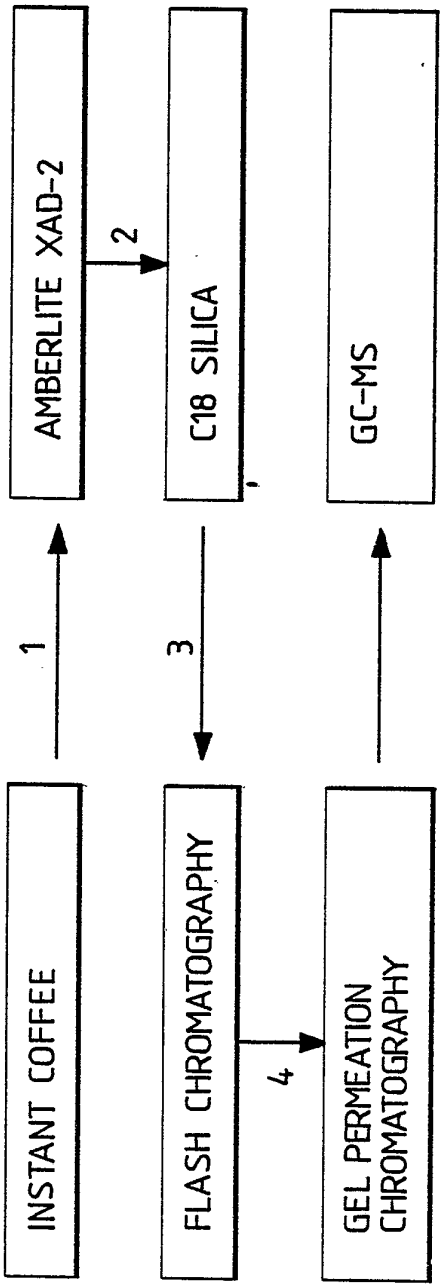

INSTANT COFFEE
AMBERLITE XAD-2
C18 SILICA
GC-MS
FLASH CHROMATOGRAPHY
GEL PERMEATION CHROMATOGRAPHY
1
2
3
4

FIG 1

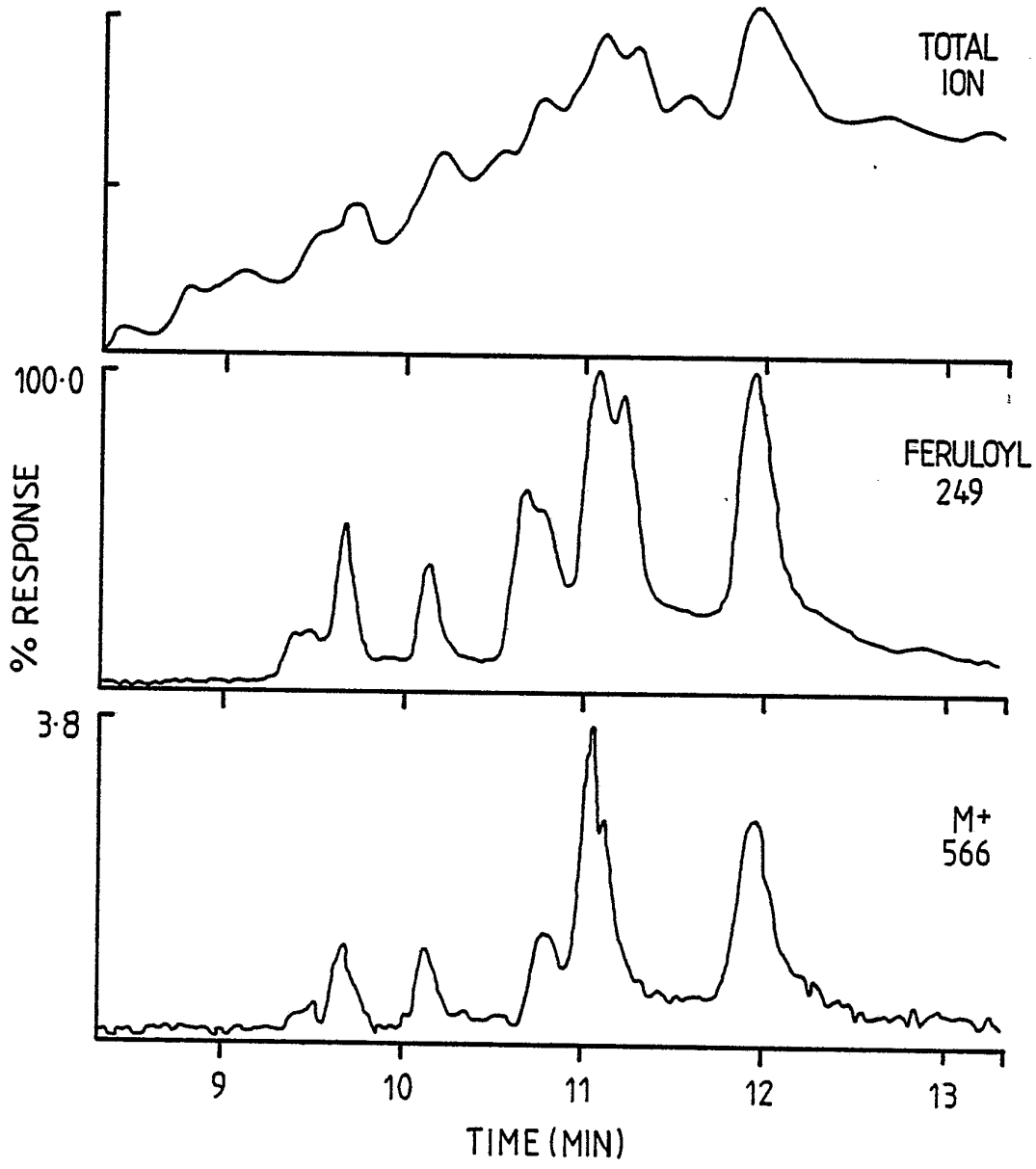

TOTAL
ION
100·0
% RESPONSE
FERULOYL
249
3·8
M+
566
9
10
11
12
13
TIME (MIN)

FIG 2A

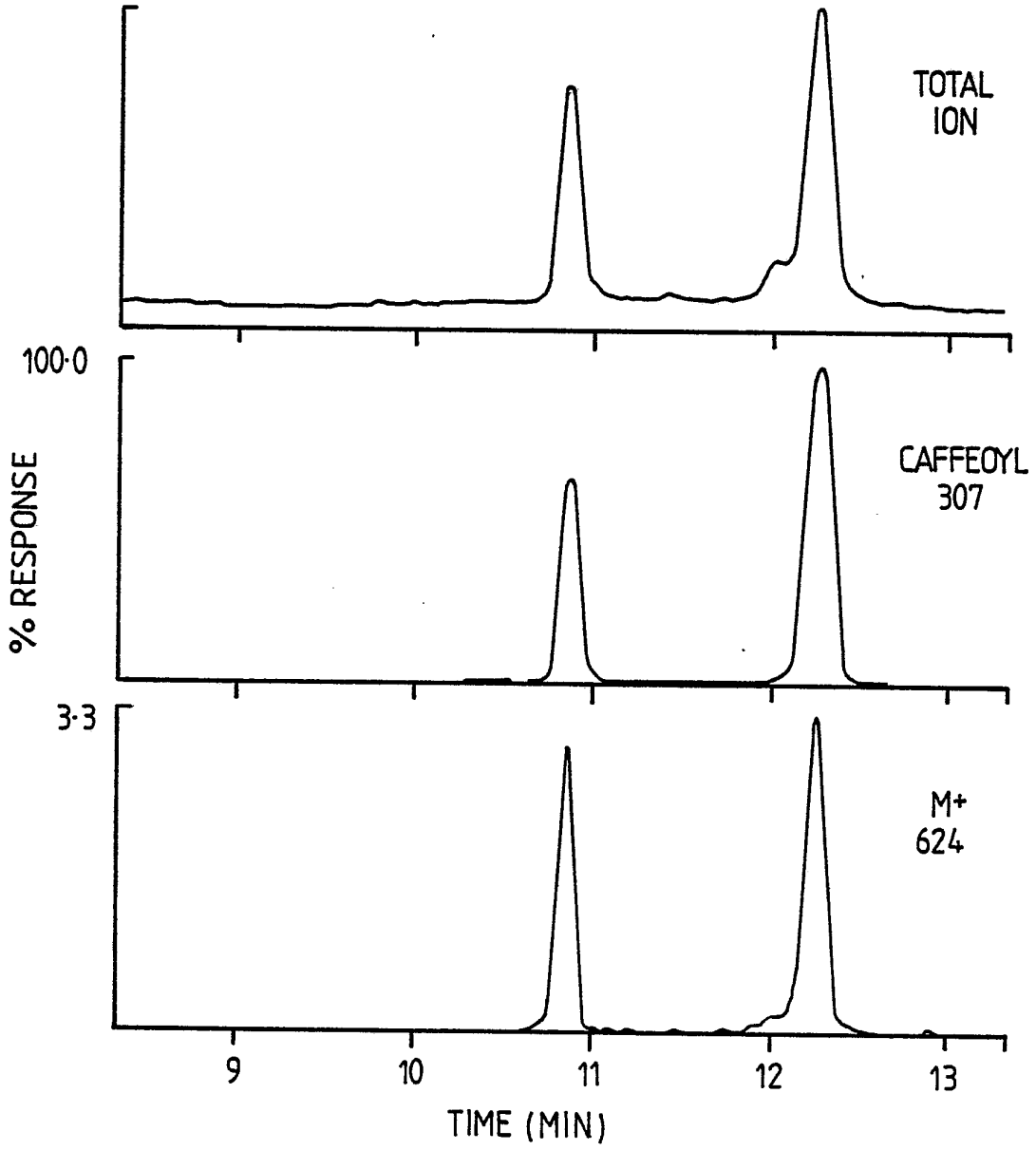

TOTAL ION
100·0
CAFFEOYL 307
% RESPONSE
3·3
M+ 624
9
10
11
12
13
TIME (MIN)

FIG 2B

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 85/00200

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[4] C07D 307/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	C07D 307/00
US Cl.	549/302

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

AU : IPC as above; Australian Classification 09.62-20, 09.171.0

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No [13]
X	AU,B, 9158/55 (206150) (FARMACEUTICI ITALIA S.A.) 17 November 1955 (17.11.55)	(1,2,4-6)

* Special categories of cited documents. [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
22 October 1985 (22.10.85)	(28.10.85) 28 OCTOBER 1985
International Searching Authority	**Signature of Authorized Officer**
Australian Patent Office	per C.A. BRICK

Form PCT/ISA/210 (second sheet) (January 1985)