Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/1396
DOI URL: http://dx.doi.org/10.21474/IJAR01/1396
ISSN NO. 2320-5407

RESEARCH ARTICLE

Isolation and Characterization of a Prenylated Chalcone from Libyan *Pistacia lentiscus* L. Leaves

***Abdel Karim. M.[1], Kutaiba. I[2], Mongid. S.[3] and Hunida. E.[4].**

1. Sudan University of Science and Technology, Faculty of Science.
2. Faculty of Medical Technology, Department of Drug Technology, Derna, Libya.
3. University of Bahri, College of Applied and Industrial Sciences.
4. Shaqra University ,Faculty of Education.

Manuscript Info

Manuscript History

Received: 12 June 2016
Final Accepted: 15 July 2016
Published: August 2016

Key words:-
Pistacia lentiscus , Isolation,Characterization,Chalcone.

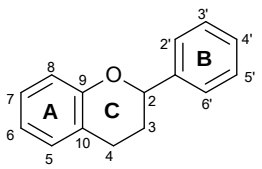
Abstract

A prenylated chalcone was isolated from the ethanolic extract of *Pistacia lentiscus* leaves by preparative thin-layer chromatography technique. The structure of the isolate was elucidated via a combination of sensitive spectral tools including : UV, IR, 1D and 2D NMR and high-resolution mass spectrometry to be a : 2',4',4-trihydroxy-6'-methoxy-3'-prenylchalcone.

Copy Right, IJAR, 2016,. All rights reserved.

Introduction:-

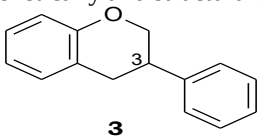
Flavonoids are phenolic substances isolated from a wide range of vascular plants, and more than 8150 different flavonoids have been reported(Harborne,1989) . Flavonoids are located inside the cells or on the surface of various plant organs and have various functions in plants(Marais *et.al.*,2006) . They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellents, and for light screening(Pieatta,2000) . Many studies have shown that flavonoids exhibit biological and pharmacological activities, including antioxidant, cytotoxic, anticancer, antiviral, antibacterial, cardioprotective, hepatoprotective, neuroprotective, antimalarial, antileishmanial, antitrypanosomal and antiamebial properties(Harborne and Williams,2000; Nowakowska,2007 ;Williams *et.al*.1999;Weimann *et.al*.,2002;Havsteen,2000) . These biological and pharmacological properties are usually attributed to their free radical scavenging efficacies, metal complexion capabilities, and their ability to bind to proteins with a high degree of specificity(Snow *et.al.*,2005) .The basic flavonoid structure contains the flavan nucleus (**1**), which consists of 15 carbon atoms derived from a C_6-C_3-C_6 skeleton. A flavonoid skeleton is composed of two aromatic rings (commonly designated as A and B), which are linked by a three-carbon chain. The connecting carbon chain combines with an oxygen to form a heterocyclic central C-ring for most flavonoids with the exception of chalcones (**2**) in which the carbon chain between the A and B rings is linear(Beecher,2003) . The numbering scheme for chalcones differs from three-ring flavonoids in that the A ring, rather than the B ring carbons are labeled as prime.



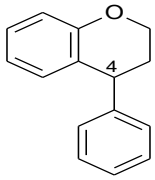
1 **2**

Corresponding Author:- Abdel Karim. M.
Address:- Sudan University of Science and Technology, Faculty of Science.

Depending on the position of the linkage of the aromatic B-ring to the benzopyrano (chromano) moiety, this group of natural products may be divided into three classes: the flavonoids (2-phenylbenzopyrans) **1**, isoflavonoids (3-phynylbenzopyrans) **3**, and the neoflavonoids (4-phynylbenzopyrans) **4**. These groups usually share a common chalcone precursor, and therefore are biogenetically and structurally related(Marais *et.al.*,2006).

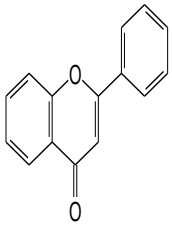


3

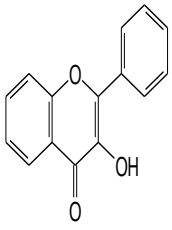


4

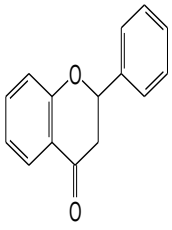
Based on the degree of oxidation and saturation present in the heterocyclic C-ring, the flavonoids may be divided into the following groups: flavone s(**5**), flavonols (**6**), flavanone s(**7**), dihydroflavonols (**8**), flavan s(**1**), flavan-3-ols (**9**), flavan-4-ol s(**10**), flavan-3,4-diol s(**11**), and anthocyanidins (**12**).



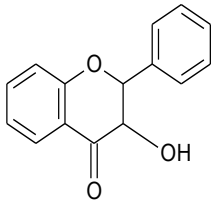
5



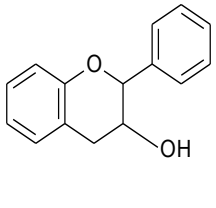
6



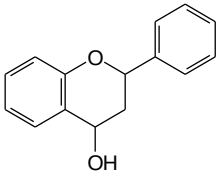
7



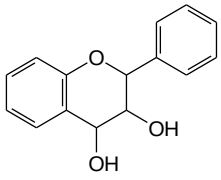
8



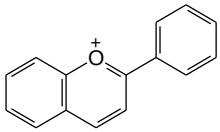
9



10

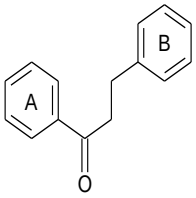


11

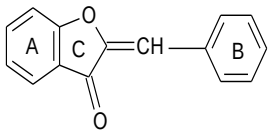


12

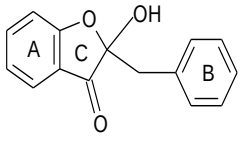
Natural products such as chalcones (**2**), dihydrochalcones (**13**), aurones (**14**) and auronols (**15**) also contain a C_6-C_3-C_6 backbone and are considered to be minor flavonoids(Williams *et.al.*,1999) .



13



14



15

The flavonoids may be modified by hydroxylation, methoxylation, or *O*-glycosylation of hydroxyl groups as well as *C*-glycosylation directly to carbon atom of the flavonoid skeleton. In addition, alkyl groups (often prenyls) may be covalently attached to the flavonoid moieties, and sometimes additional rings are condensed to the basic skeleton of the flavonoid core. Flavonoid glycosides are frequently acylated with aliphatic or aromatic acid molecules. These derivatives are thermally labile and their isolation and further purification without partial degradation is difficult. Condensed tannins create a special group of flavonoid compounds formed by polymeric compounds built of flavan-3-ol units, and their molecular weights often exceeding 1,000 Da(Stobiecki and Kachlicki ,2006) .

Pistacia lentiscus L. is an evergreen shrub or small tree growing to 1 – 8 m tall(Iauk *et.al.*1996) with a long tradition in folk-medicine since the ancients Greeks(Palevitch and Yaniv,2000) . The aerial part has traditionally been used as a stimulant, for its diuretic properties, and to treat hypertension, coughs, sore throats, eczema, stomach aches, kidney stones and jaundice(Palevitch and Yaniv,2000; Bentley and Trimen,1980). Biological studies have also indicated that *P. lentiscus* has antifungal(Kordali *et.al*.2003) antimicrobial(Magiatis *et.al.*1999), antioxidant(Benhammou *et.al.*,2007),anticancer(Balan,2007) and cytotoxic(Cvitanovic and Marusic ,1994) activities.

In our search for structurally and biologically interesting constituents of Libyan plants, this work was designed to investigate the flavonoids of the medicinally import species: *Pistacia lentiscus.*

Results and Discussion:-

Compound **I** was isolated as a yellow crystalline needles from ethanolic extract of the leaves of *Pistacia lentiscus*. The ethanolic extract of the plant was subjected to preparative TLC on silica gel and the TLC plates were developed with the solvent system: *n*-BuOH–HOAc–H_2O (5:1:4) to obtain compound **I**. The structure of compound **I** was elucidated on the basis of MS, IR, UV, ^{1}H NMR and ^{13}C NMR spectroscopic data, including 2D NMR experiments.

The IR spectrum of compound **I** showed characteristic absorption bands at υ (KBr) : 3423 (OH), 2925 (CH-aliph. stretching), 1625 (C=O), 1560 and 1475 (C=C, Ar), 1228 (C-O) cm^{-1}. The presence of conjugated carbonyl at 1625 cm^{-1} indicated that compound **I** belongs to: flavones, flavonols, chalcones or aurones(Harborne,1989).

The UV spectrum of compound **I** in MeOH showed characteristic dominant absorption band at 370 (Band I) and a diminished absorption band at 240 (Band II) nm, indicating the presence of a chalcone skelton (Mabry *et.al*,1970).

The chalcone skelton of compound **I** can easily be distinguished from the remaining classes of flavonoids by the two set of doublets at δ_H 7.79 and 7.67 (each 1H, d, *J*=15.5 Hz) in the ^{1}H-NMR spectrum and its corresponding carbons resonating at δ_C 125.9 and 143 in the ^{13}C-NMR spectrum assigned to α- and β-carbon atoms respectively(Mabry et.al,1970; Kitanaka and Takido,1992; Nikolov *et.al.*1980; Agrawal,1989) .

The ^{1}H NMR spectrum of compound **I** showed a number of signals characteristic of chalcones and isopentenyl moiety(Agrawal,1989; Markham,1982; Markham,1975) . The spectrum also showed a double doublet resonating at δ 7.49 ($J_{2,3/6,5}$ = 8.5 Hz) and 6.82 ($J_{3,2/5,6}$ = 8.5 Hz) due to the aromatic H-2,6 and H-3,5, respectively, of the B-ring and their coupling constants suggested *ortho* coupling.

Table1:- ^{1}H and ^{13}C NMR data of compound **I**

Position	δ_H	δ_C
C=O		194.1
α	7.79 d (15.5)	125.9
β	7.67 d (15.5)	143.3
1		128.5
2,6	7.49 d (8.5)	131.3
3,5	6.82 d (8.5)	116.9
4		161.1
1'		106.5
2'		166.2
3'		109.4
4'		163.7
5'	6.02 s	91.6
6'		162.4
1"	3.23 d (7.2)	22.3
2"	5.20 t	142.3
3"		131.4
4"	1.76 s	26.0

the 4-position of the B-ring is oxygenated(Mabry *et.al.*,1970). A singlet at δ 3.89 (s, 3H) is due to a methoxy function. Another singlet resonating at δ 6.02 (s, 1H) was assigned to H-5'. The presence of prenyl (isopentenyl) unit was indicated by typical chemical shifts and *J* values(Nkengfack *et.al.*,1989). The H-2″ olefinic proton signal of isopentenyl unit was observed as a triplet at δ 5.20 (1H, t, *J* = 7.2 Hz), while the two methyl signals typically appeared as singlets at δ 1.76 and δ 1.65ppm each integrating for 3 protons. They each have a unique shape due to

extensive long-range coupling. The signal corresponding to the methylene 1″ protons appeared as one broad isochronic (2H) doublet at δ 3.23 with *J* = 7.2 Hz.

The ^{13}C-NMR spectrum of compound **I** showed a pattern characteristic of flavonoids with three methyls, one methylene, eight methines and nine quaternary carbons in the molecule. The chalcone skeleton of **I** was further supported by the presence of two characteristics olefinic carbons resonating at δ_C 125.9 (α-C) and 143.3 (β-C). The most downfield quaternary signal at δ_C 194.1 was assigned to the ketonic carbonyl carbon . The downfield chemical shift of the C=O indicated the presence of an *O*-methy group at the adjacent C-6' in A-ring(Agrawal ,1989). Other signals at : δ_C 106.5 (C-1'), 166.2 (C-2'), 109.4 (C-3'), 163.7 (C-4'), 91.6 (C-5'), 162.4 (C-6'), 22.3 (C-1''), 124.3 (C-2''), 131.4 (C-3''), 26.0 (C-4'') and 17.9 (C-5'') further supported the presence of a chalcone skeleton and an isoprenyl- substituted A-ring . The B-ring carbons resonated at δ_C 128.5 (C-1), 131.3 (C-2,6), and 116.9 (C-3,5), indicating a C-4 substituent group. The methyl carbon signal at δ_C 56.2 (C-6') indicated the presence of *O*-methyl group in the molecule. The identity of the isoprenyl unit was ascertained through comparison of the chemical shift of its carbon with standard reference data(Nkengfack *et.al*.,1989). A careful study of the ^{13}C-NMR data and its comparison with the reported data again indicated that the aglycone was a chalcone(Stevens *et.al*,1997). The complete ^{13}C-NMR and multiplicity data of compound **I** are displayed in Table 1.

The HSQC technique is used to establish the direct one-bond ^{1}H-^{13}C connectivities. The protons of rings A, B and C i.e. H-5' (δ_H 6.02), α-H (δ_H 7.79), β-H (δ_H 7.67), H-2,6 (δ_H 7.49), H-3,5 (δ_H 6.82) and 6'-*OCH*$_3$ (δ_H 3.90) showed one-bond correlations with C-5' (δ_C 91.6), α-C (δ_C 125.9) and β-C (δ_C 143.3), C-2,6 (δ_C 131.3), C-3,5 (δ_C 116.9) and 6'-*OCH*$_3$ (δ_C 56.2), respectively . Similarly protons signals of the isopentenyl moiety, i.e. H-1" (δ_H 3.23), H-2" (δ_H 5.20), H-4" (δ_H 1.76) and H-5" (δ_H 1.65) also showed direct connectivities with C-1" (δ_C 22.3), C-2" (δ_C 124.3), C-4" (δ_C 26.0) and C-5" (δ_C 17.9) respectively.

The ^{1}H-^{1}H COSY NMR spectrum of compound **1** showed couplings between the doublets of H-2,6 (δ_H 7.49), H-3,5 (δ_H 6.82) of aromatic B-ring . The C-α olefinic proton (δ_H 7.79) showed correlation with the C-$_\beta$ olefinic proton (δ_H 7.67). Interactions of the C-1" methylene protons (δ_H 3.23) with the C-2" methine proton (δ_H 5.20) was observed. The latter (H-2") showed coupling with the C-4" methyl protons (δ_H 1.76), which was in turn coupled with the C-5" methyl protons (δ_H 1.65). The C-1" methylene protons showed correlation with the C-4" methyl protons (δ_H 1.76), which was in turn coupled with the C-5" methyl protons (δ_H 1.65).

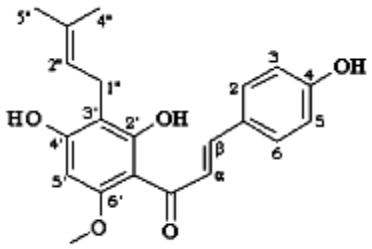
Long-range ^{1}H-^{13}C correlations were determined from the HMBC experiment which helped in connecting various structural fragments and in assigning the chemical shifts of quaternary carbons. The H-5' (δ_H 6.02) showed two-bond couplings with C-4' (δ_C 163.7) and C-6' (δ_C 162.4), three-bond couplings with C-3' (δ_C 109.4) and C-1' (δ_C 106.5). The H-2,6 (δ_H 7.49) of aromatic B-ring showed coupling with C-2,6 (δ_C 131.3), C-3,5 (δ_C 116.9), C-4 (δ_C 161.1) and β-C (δ_C 143.3), while H-3,5 signal (δ_H 6.82) was found to be coupled with C-3,5 (δ_C 116.9), C-4 (δ_C 161.1) and C-1 (δ_C 128.5).

The α-H (δ_H 7.79) showed couplings with C-1 (δ_C 128.5), β-C (δ_C 143.3) and C=O (δ_C 194.1), while β-H (δ_H 7.67) signal was found to be coupled with C-2,6 (δ_C 131.3), α-C (δ_C 125.9) and C=O (δ_C 194.1). The O-methyl protons (δ_H 3.90) were found to be coupled with C-6' (δ_C 162.4), which indicated that the O-methyl group was present at C-6' of A-ring. The attachment of the isopentenyl moiety at C-3' of A-ring was established by the HMBC interaction of methylene H-1" (δ_H 3.23) with C-3' (δ_C 109.4) of aglycone. The H-1" (δ_H 3.23) showed two-bond couplings with C-3' (δ_C 109.4) and C-2" (δ_C 124.3), and three-bond couplings with C-2' (δ_C 166.2) and C-4' (δ_C 163.7). The H-4" (δ_H 1.76) showed couplings with C-5" (δ_C 17.9), C-3" (δ_C 131.4) and C-2" (δ_C 124.3), while H-5" (δ_H 7.67) signal was found to be coupled with C-4" (δ_C 26.0), C-3" (δ_C 131.4) and C-2" (δ_C 124.3).

The high-resolution mass spectrum of **(I)** showed a $[M-1]^-$ ion at 353.1390 corresponding to a molecular formula of $C_{21}H_{22}O_5$. The electrospray ionization mass spectrum (ESI-MS) of **(I)** showed several fragments characteristic of an isopentenylated chalcone skeleton. The major peaks at *m/z* 233 (A- fragment) and 119 (B- fragment) were due to the cleavage of ring C through a retro-Diels Alder mechanism (Scheme I), and indicated the presence of an isopentenyl unit and methoxy group on ring A and a hydroxyl function on ring B of the aglycone(Markham ,1982 ; Markham ,1975).

On the basis of the above cumulative spectral data, the structure of compound **I** was deduced to be a: 2`,4`,4-trihydroxy-6`-methoxy-3`-prenylchalcone(**I**).

.



I

Materials and Methods:-

Materials:-

General Experimental Procedures:-

UV spectra (Shimadzu UV-1203) were recorded in MeOH, whereas IR spectra (Nicolet 510P FT-IR) were obtained as a KBr disc film. ^{1}H NMR (Bruker AM-500, 500 MHz) and ^{13}C NMR (Bruker AC-200, 75 MHz) spectra were acquired in MeOH-d_4 with TMS as internal standard, whereas EIMS (Shimadzu QP-5000/Gc-17A/DI-50) and HREIMS (VG-ZAB-VSEQ) were recorded at 70 eV (ionizing potential) using a direct inlet system. To monitor the preparative separations, analytical thin-layer chromatography (TLC) was performed at room temperature on precoated 0.25 mm thick silica gel 60 F254 glass plates (20 x 20 cm). Chromatograms were visualized after drying (i) by UV light and (ii) by a phenol specific spray reagent, $FeCl_3$ (3% in dry ethanol). All other chemicals and reagents were analytical grade.

Plant Material:-

Leaves of *Pistacia lentiscus* were collected from "Jebal Akhdar" mountains (Libya), in February 2015. The plant was identified by the Botany Department, Omar Almuktar University, and voucher specimens were deposited in the herbarium of that Department. The plant sample was shade-dried and ground into uniform powder.

Extraction and Isolation:-

Shade-dried leaves of *Pistacia lentiscus* (1Kg) were macerated with 95% ethanol (3L) at room temperature for 2 days. The extract was filtered, and the solvent was removed under reduced pressure at relatively low temperature (<35^{o}C) to leave a dark green solid (54 g).

Silica gel 60F_{254} and water were mixed to form a slurry which was spread over clean glass plates. These plates were used without activation. Small amount of the crude product of *Pistacia lentiscus* was dissolved in 95% ethanol and applied as concentrated spots on silica gel plates. Many solvent systems have been employed for the separation of flavonoids using TLC. However, the solvent system that achieved the best separation was: *n*-BuOH–HOAc–H_2O, (5:1:4). Only one major spot was detected by spraying with 3% solution of ferric chloride $FeCl_3$ in ethanol.

The crude product(*ca*.0.5g) of *Pistacia lentiscus* was dissolved in the minimum amount of ethanol and applied on (20 x 20 cm) silica gel plates as a narrow strip. The plates were developed with the solvent system: *n*-BuOH–HOAc–H_2O, (5:1:4) and the chromatograms were located under UV light. The sole major band was scratched off and the product eluted from silica gel with ethanol. After the usual workup, a solid (**compound I**) was obtained.

Compound I :2',4',4-trihydroxy-6'-methoxy-3'-prenyl chalcone : yellow crystalline needles (*n*-BuOH–HOAc–H_2O, 5:1:4); mp 229-230 °C; IR (KBr) ν_{max} 3423 (OH), 2925 (CH-stretching), 1625 (C=O), 1560 and 1475 (C=C, Ar), 1228 (C-O) cm^{-1}; UV (MeOH) λ_{max} (log ε) 369 (1.85), 270 (0.75) nm; ^{1}H , ^{13}C NMR (MeOH-d_4, 500 MH_Z and MeOH-d_4, 125 MH_Z, respectively); ESIMS *m*/*z* 353 [M - H]$^{-}$; HRESIMS *m*/*z* 353.1390 [M - H]$^{-}$ ..

References:-

1. **Agrawal**, P.K., *Carbon-13 NMR of Flavonoids*, Elsevier Science Publishers, Amsterdam, 1989.
2. **Benhammou**, N., Atik, B., Kadifkova, P., *Adv. Food Sci*., **29**, 155 (2007).
3. **Balan,** K.V. *et al*., *PhytoMed.*, **14**, 263 (2007).
4. **Beecher,** G.R., *J. Nutr*., **133**, 3248S (2003).
5. **Bentley,** R.Y., Trimen, H., *Food Chem.*, **4**, 1 (1980).
6. **Cvitanovic**, S., Marusic, M., *J Investig Allergol Clin Immunol*., **4**, 96 (1994).
7. **Harborne,** J.B., and Williams, C.A., *Phytochemistry*, **55**, 481 (2000).
8. **Harborne**, J.B., General procedures and measurement of total phenolics, in *Methods in Plant Biochemistry, Vol. 1, Plant Phenolics*, Harborne, J.B., Ed., Academic Press, London, 1989, chap. 1.
9. **Havsteen**, B.H., *Pharmacol. Therapeutics*, **96**, 67 (2000).
10. **Iauk,** L., Ragusa, S., Rapisarda, A., Franco, S., Nicolosi, V.M., *J. Chemother*., **8**, 207 (1996).
11. **Kordali,** S., Cakir, A., Zengin, H., Duru, M.E., *Fitoterapia.*, **74**, 164 (2003).
12. **Kitanaka,** S., and Takido, M., *Chem. Pharm. Bull*., **40**, 249 (1992).
13. **Magiatis** P, Melliou E, Skaltsounid AL, Chinou IB, Mitaku, S., *Plant Med*., **65**, 749 (1999).
14. **Mabry**, T.J., Markham, K.R., and Thomas, M.B., *The Systematic Identification of Flavonoids*, Springer-Verlag, New York, 1970.
15. **Markham,** K.R., "*Techniques of Flavonoids Identification*", Academic Press. London, 1982.
16. **Markham**, K., Isolation techniques for flavonoids, in *The Flavonoids*, Harbone, J.B., Mabry, T.J., and Mabry, H., Eds., Academic Press, New York, 1975.
17. **Marais,** J.P.J., Deavours, B., Dixon, R.A., and Ferreira, D., The stereochemistry of flavonoids, in *The Science of Flavonoids*, Grotewold, E., Ed., Springer Science, New York, 2006, chap. 1.
18. **Nkengfack**, A.E., Sanson, D.R., Tempesta, M.S., and Fomum, Z.T., *J. Nat. Prod.*, **52**, 320 (1989).
19. **Nikolov**, N., Seligmann, O., Wagner, H., Horowitz, R.M., and Gentili, B., *Planta Medica*, **44**, 50 (1980).
20. **Nowakowska**, Z., *Eur. J. Med. Chem.*, **42**, 125 (2007).
21. **Palevitch**, D., Yaniv, Z., *J. Ethnopharmacol*, **1**,198 (2000).
22. **Pieatta**, G.P., Flavonoids as antioxidant, *J. Nat. Prod*., **63**, 1035 (2000).
23. **Stevens**, J.F., Ivancic, M., Hsu, V.L., and Deinzer, M.L., *Phytochemistry*, **44**, 1575 (1997)
24. **Snow,** R.W., Guerra, C.A., Noor, A.M., Myint, H.Y., and Hay, S.I., *Nature*, **434**, 214 (2005).
25. **Stobiecki**, M., Kachlicki, P., Isolation and identification of flavonoids, in" The Science of Flavonoids", Grotewold, E., Ed., Springer Science, New York, 2006, chap. 2
26. **Williams,** C.A., Harborne, J.B., Geiger, H., and Hoult, J.R.S., *Phytochemistry*, **51**, 417 (1999).
27. **Weimann,** C., Goransson, U., Ponprayoon-Claeson, P., Bhlin, L., Rimpler, H., and Heinrich, M., *J. Pharm. Pharmacol*., **54**, 99 (2002).