Research Article

Eugenol Derivatives as Future Potential Drugs

Pramod K. Awasthi[1], Satish C. Dixit[*1], Nidhi Dixit[1], Anoop K. Sinha[1]

[1]Dapartment of Chemistry,D.B.S College, Kanpur,208006,India

[2]Central Drug Research Institute, Lucknow, India

*For correspondence:*S. C. Dixit, Dapartment of Chemistry,D.B.S College, Kanpur,208006,India

*E-mail:*scdixit17@rediff.com

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ABSTRACT

Eugenol, a phytochemical , is a pale yellow oil which is isolated from essential oil of *Ocimum sanctum* Rama. Eugenol has wide medicinal applications. It affects central nervous system and cardiovascular system. It is used in dentistry and as an analgesic, antipyretic, antimicrobial, anti septic, antidepressant agent. Its analogues also show many biological activities which prompted us to synthesize new analogues for their future application as bioactive molecules.

Key words: Eugenol, Essential Oil, *O.sanctum,* ester derivative

INTRODUCTION

Eugenol, 2-methoxy-4-(2-propenyl) phenol is a phytochemical obtained from *Syzigium aromaticum, Ocimum Sanctum*, etc. The worldwide production of eugenol is estimated to approximately 22000 Kgs. It finds wide applications ranging from perfumeries, flavourings, and in medicines. Eugenol affects the peripheral aspects of the cardiovascular system. The heart is not the principal site of action since eugenol has little effect on the electrical activity and only slightly reduces the contractile force unless a little fatal dose in used [1]. The intravenous injection of eugenol (upto 0.5 ml) to mongrel dogs caused a drop in arterial blood pressure. Increased blood flow observed after intraarterial and intravenous injections suggests that blood vassels are the main site of action within the cardiovascular system. Eugenol affects the central nervous system. It is an anaesthetic in mice and dogs. Larger doses lengthen the sleeping time [2]. Eugenol is used in combination with zinc oxide as a surgical dressing [3], pulp capping agent [4] cavity liner,temporary cement [5] in mouth washes and endodontic therapy [6] in the study of mucous secretions and in gastric cytology [7-9].It shows has an antidepressant[10] like activity, antibacterial [11] and antioxidant [12-14] activity. It reduces body temperature in rats at doses exceeding 50 mg/kg. It is a general acting antimicrobial and antianimal toxin with analgesic properties for humans [15-17]. To date there has been no evidence to demonstrate a significant carcinogenic effect of eugenol in any species [17]. The use of eugenol derivatives as general anesthetic agents has been explored [3].The above mentioned varied important medicinal applications prompted us to isolate eugenol from essential oil of *O. sanctum* Rama and synthesize its some new ester based derivatives of which may prove great potential future drugs against many diseases.

RESUTLS AND DISCUSSION

NMR Spectroscopic investigations

Eugenol was obtained as a yellow viscous compound. The ^{1}H NMR spectrum of major constituent of O. sanctum Rama leaf oil showed the presence of 12 protons in the molecule. The presence of 3 downfield proton at ? 6.74, 6.76, 6.92 ppm indicated the presence of an aromatic ring in the molecule which is substituted at 3 position. The presence of a singlet for 3 protons at ? 3.86 suggested the presence of a methoxy group on the aromatic ring. Further the presence of doublet at ??3.38 for 2 protons suggested the presence of a -CH_2 attached to an aromatic ring. The presence of downfield

doublet at ? 5.12 and 5.19 (1 H each) further suggested the presence of an exocyclic double bond which was supported by the presence of one proton multiplet at ? 5.96 for a methine proton. The ^{13}C NMR spectrum of PKA 100 showed the presence of 10 carbon atoms in the molecule. Further its DEPT 90^0 and DEPT 135^0 showed the presence of three quaternary, four tertiary, two secondary and one methyl carbon in the molecule. Since from the ^{1}H NMR out of three aromatic proton one proton is singlet at ? 6.74 and remaining two aromatic proton at ? 6.76 and 6.96 are doublets suggesting 1, 2 and 4 substitution on the aromatic ring. Further the downfield quaternary carbon at ? 144.3 and 146.9 should be assign to aromatic carbon (1 and 2) bearing a hydroxyl and methoxy group while the upfield quaternary carbon at ? 138.1 for allylic group at position 4.

The reaction product of eugenol and palmitoyl chloride, on work-up and purification gave a white crystalline compound. The ^{1}H NMR spectrum of PKA-101 showed presence of 42 protons in the molecule. The ^{13}C NMR specturm of PKA-101 showed presence of 26 intense carbon signals instead of 10 indicating addition of palmitate group in PKA 101. This was also proved from the ^{13}C NMR spectra the downfield carbon signal at ??172.0 ppm confirm the presence of ester group in the molecule.

Similarly the presence of CH_2 protons between ??2.56 (2H) and ? 1.75 (2H) in ^{1}H NMR could be assigned to H-2' and H-3' respectively, which were supported by ^{13}C NMR signals at ??34.0 t and 25.0t ppm respectively. The presence of twelve methylene groups were proved by the downfield signal at ? 1.26 (12H, br s) in ^{1}H NMR, which could be assigned to H-4' to H-15'. The above assignment of twelve methylene groups were supported by twelve methylene ^{13}C NMR signals between ? 29.0t to 22.7t ppm respectively. The upfield br s at ??0.87 (3H, br s) in the ^{1}H NMR was assigned to H-16', which was also supported by ^{13}C NMR signals at ??14.1q ppm for methyl of palmitoyl (C-16') group.

The reaction product of eugenol and p-anisoyl chloride, on work up and purification, gave the grayish crystalline compound PKA-102. The ^{1}H NMR spectrum of PKA-102 showed presence of 18 protons in the molecule. The ^{13}C NMR spectrum of PKA-102 showed presence of 18 intense carbon signals instead of 10 indicating the addition of p-anisoate group. This was also proved by the presence of deshielded carbon signal at ? 164.5 ppm. Similarly the presence of 4 aromatic protons at ? 7.05 (2H, d, J-7.65 Hz, H-3'' and H-5'') and 8.16 (2H, d, J-8.11Hz, H-2'' and H-6''), other than eugenol signals indicated that the remaining two positons of benzene ring are substituted by -OCH_3 and an ester group respectively. The appearance of a downfield three protons singlet at ? 3.87 clearly indicated the presence of a methoxy group in the benzene ring.

Further confirmation of p-anisoyl group in PKA-102 was made from ^{13}C NMR spectrum, which showed six intense additional carbon signals, other than those of eugenol (PKA-100). These six extra carbon signals were distinguished by DEPT into three quaternary signals two methine and one methyl carbons. Out of three quaternary signals, the downfield quaternary signal at 164.5s ppm, confirmed the presence of an ester linkage. While the the other quaternary signal at 163.7s ppm suggested the presence of a -OCH_3 substitution in benzene ring. The third quaternary carbon at 138.0s ppm could be assigned to the C-1'' of benzene ring bearing an ester group.

Keeping the above substitution in mind the remaining 4 protons at ? 7.05 and 8.16 (2H each) could be assigned to H-3'' & H-5'' and H-2'' & H-6'' respectively. The above assignment of four aromatic protons were also supported by ^{13}C NMR methine signals at ? 132.3d and 112.8d ppm for C-2'' & C-6'' and C-5'' & C-6'' carbons respectively. The methyl signals at ? 55.8q ppm in DEPT-135^0; was assigned to -OCH_3 at para position of benzene ring.

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The reaction product of eugenol and myristoyl chloride, on work up and purification gave the white crystalline compound. The ^{1}H NMR spectrum of PKA-103 showed presence of 38 protons in the molecule. The ^{13}C NMR spectrum of PKA-103 showed the presence of 24 intense carbon signals instead of 10 indicating the addition of myristate group in the molecule.

. This was also proved from the ^{13}C NMR spectra in which deshielded signal at ? 172.0q confirm the presence of ester group in the molecule. Similarly the presence of CH_2 protons between ? 1.75, 2.56 and CH_3 protons at ? 0.87 ppm respectively other than eugenol were also observed.

The two protons downfield signals of ? 2.56 (2H) and 1.75 (2H) in ^{1}H NMR could be assigned to H-2' and H-3' respectively, which were supported by ^{13}C NMR signals at ? 34.0 and 25.0 ppm respectively. The presence of ten methylene groups was proved by the downfield signal at ? 1.26 (20H, br s) in the ^{1}H NMR of PKA-103, could be assigned to H-4' to H-13'. The up field br s at ? 0.88 (3H, br s) in the ^{1}H NMR could be assigned to H-14', which was

also supported by ^{13}C NMR signal at ? 14.3q ppm for methyl of myristoyl (C-14') group.

The reaction product of eugenol and lauroyl chloride, on reaction work up and purification gave gray colour crystalline compound. The ^{1}H NMR spectrum of PKA-104 showed presence of 34 protons in the molecule. The ^{13}C NMR spectrum of PKA-104 showed presence of 22 intense carbon signals instead of 10 indicating addition of laurate group in PKA-104..

This was also proved from the ^{13}C NMR which clearly showed shifting of Carbon atom at C-1 from ? 144.3s to 138.78d ppm respectively. Similarly the presence of CH_2 protons between ? 2.54, 1.75 and CH_3 protons at ? 0.88 respectively other than eugenol were also recorded.

The two protons downfield signals at 2.54 (2H) and 1.75 (2H) in ^{1}H NMR could assigned to H-2' and H-3' respectively, which were supported by ^{13}C NMR signals at ? 34.4t and 25.4t ppm respectively. The presence of eight methylene groups were proved by the downfield signal at ? 1.27 (16H, br s) in ^{1}H NMR, which could be assigned to H-4' to H-11'. The above statement of eight methylene groups were supproted by eight methylene ^{13}C NMR signals between 22.9 to 29.4 respectively. The upfield signals at ? 0.88 (3H) in the ^{1}H NMR was assigned to H-12', which was further supported by ^{13}C NMR signals at 14.4q ppm for methyl of laurate (H-12') group.

EXPERIMENTAL

Methods

Plant material

The O. sanctum Rama was obtained from Chandra Shekhar Azad University of Agricultural and Technology, Kanpur (India) in the month of July 2005.

Isolation of the oil.

The essential oil of O. sanctum Rama was isolated by hydrodistillation in a Clevenger type apparatus for 4 hrs. The essential oils was dried over anhydrous Sodium Sulphate and stored in glass vials. The oil was stored in refregerator, till analysis.

Analysis of the oil:

The essential oil thus isolated was analysed using GC and GC-MS analysis.

(i) Gas Chromatography (GC)

The oils were analysed on a Hewlett-Packard 5980 A gas Chromatograph equipped with a fused silica capillary column (50 X 0.25mm) coated with methyl silicon (thickness 0.17mm) with FID detector. GC conditions were: nitrogen as carrier gas (1ml/min), split ration 1:75, injection temperature 250°C, FID temp. 300°C and programmed from 80°C to 200°C at a rate of 2°C/min. The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes

(ii) GC/MS analysis

GC/MS data were obtained on a Perkin Elmer Turbo Mass spectrometer instrument using a PE-WAX column (60m X 0.32mm, film thickness 0.25µm). Temperature programmed; 5min at 70°C, then rising at 2°C/min to 120°C and then 3°C/min from 120-240°C. Carrier gas was helium.

Identifiction of compounds

Identification of compounds was made on the basis of retention indices of the peaks with literature[18-22] values, computer matching against the Wiley and NBS liberaries spectra.

Isolation of eugenol

An essential oil of *O. Sanctum* Rama was chromatographed over a silica gel column. Elution of column was carried out with varying proportions of n-hexane and dichloromethane. Fractions eluted were subjected to preparative TLC along with standard eugenol. Bands of eugenol scratched out and adsorbed eugenol eluted with dichloromethane.

Isolation and characterization of eugenol (100)

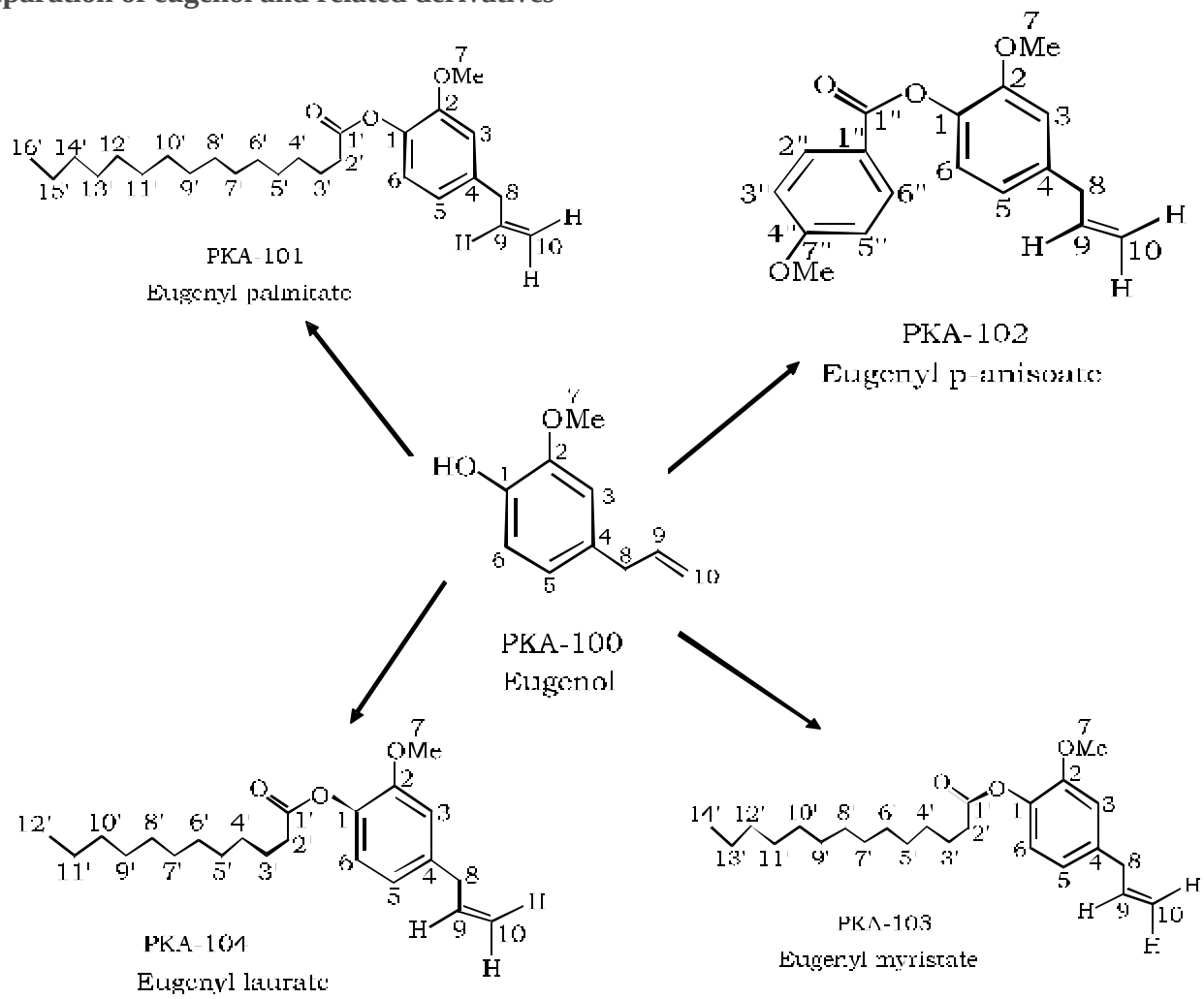
For spectroscopic analysis, yellow mass thus obtained, was dissolved in spectroscopic grade $CDCl_3$ to record NMR Spectra. Molecular formula C_{10}-$H_{12}O_2$. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.38 (2H, d, J=6.6 Hz, H-8), 3.86 (3H, s, H-7), 4.89 (1H, s, Ph-OH), 5.12 and 5.19 (1H, each d, J=19.7,H-10), 5.96 (1H, m, H-9), 6.74 (1H, s, H-3), 6.76 (1H, d, J= 8.6 Hz, H-5), 6.92 (1H, d, J= 8.6 Hz, H-6). ^{13}C NMR ($CDCl_3$, 75.5 MHz): 144.3s (C-1), 146.9s (C-2), 115.6d (C-3), 138.1s (C-4), 121.7d (C-5), 114.7d (C-6), 56.3q (C-7), 40.3t (C-8), 132.1d (C-9), 111.7t (C-10). On the basis of above 1H and 13C NMR assignments for eugenol, which was in full agreement with the reported ^{1}H and ^{13}C NMR values of eugenol.

Eugenyl palmitate (101)

To a solution of eugenol (200mg) in pyridine, palmitoyl chloride (222mg) was added .The airtight reaction mixture was continued to stirring at 130^0 on sand bath for 5h. The progress of reaction was checked by TLC. After completion of reaction ice cold water was added and the mixture was extracted with chloroform three times. The pooled chloroform extract was washed with 7% aquous solution until it was neutralized and

Scheme

Preparation of eugenol and related derivatives



dried under reduced pressure . The chloroform extract was purified by column chromatography using silica gel as adsorbent and hexane :ethyl acetate (96:4) as eluent, which resulted the white shiny crystal (150mg)with 75% yield.

For spectroscopic analysis, white mass thus obtained, was dissolved in spectroscopic grade $CDCl_3$ to record NMR Spectra. ^{1}H NMR ($CDCl_3$, 300 MHz): 0.87 (3H, t, H-16'), 1.26 (12 x 2H, br s, H-4'-15'), 1.75 (2H, m, H-3'), 2.56 (2H, t, H-2'), 3.36 (2H, d, H-8'), 3.79 (3H, s, H-7), 5.09 (2H, t, H-10), 5.94 (1H, m, H-9), 6.74 (1H, s, H-3), 6.77 (1H, d, H-5), 6.92 (1H, d, H-6). ^{13}C NMR ($CDCl_3$, 75.5 MHz): 138.7s (C-1), 150.8s (C-2), 112.7d (C-3), 138.8s (C-4), 122.5d (C-5), 120.6d (C-6), 55.7q (C-7), 40.1t (C-8), 138.8d (C-9), 116.1t (C-10), 172.0 s (C-1'), 34.0t (C-2'), 25.0t (C-3'), 29.0t (C-4'), 29.2-29.7t (C-5'-14'), 22.7 t (C-15'), 14.1q (C-16').

Eugenyl-p-anisate (102)

*T*o a solution of eugenol (200mg) in pyridine, p-anisoyl chloride (138mg) was added in 1:1.5 ratio .Some crystals of dimethylaminopyridine (DMAP)was also added as a catalyst. The airtight reaction mixture was continued to stirring at 110^0 on sand bath for 10h. The progress of reaction was checked by TLC. After completion of reaction ice cold water was added and the mixture was extracted with chloroform three times. The pooled chloroform extract was washed with 7% aquous solution until it was neutralized and dried under reduced pressure . The chloroform extract was purified by column chromatography using silica gel as adsorbent and hexane :ethyl acetate (98.5:1.5) as eluent, which resulted the white shiny crystal (144mg)with 72% yield.

^{1}H NMR ($CDCl_3$, 300 MHz): (3.39 (2H, d, J=5.79 Hz, H-8), 3.78 (3H, br s, H-7), 3.87 (3H, br s, H-7''), 4.11 (2H, t, J=18.5 Hz, H-10), 6.01 (1H, m, J=25.5 Hz, H-9), 6.78 (1H, s, H-3), 6.81 (1H, d, H-6), 6.97 (1H, d, J=8.14 Hz, H-5), 7.05 (2H, d, J= 7.62 Hz, H-3'' & 5''), 8.16 (2H, d, J= 8.11 Hz, H-2'' & 6''). ^{13}C NMR ($CDCl_3$, 300 MHz): 138.2s (C-1), 151.2s (C-2), 113.7d (C-3), 138.8s (C-4), 122.7d (C-5), 121.8d (C-6), 55.4q (C-7), 40.1t (C-8), 137.1d (C-9), 116.0t (C-10), 164.5s (C-1'), 138.8s (C-1''), 112.8d (C-2''-6''), 132.3d (C-3''-5''), 163.7s (C-4''), 55.8q (C-7'').

Eugenol myristate (103)

To a solution of eugenol (200mg) in pyridine, myristoyl chloride (200mg) was added .The airtight reaction mixture was continued to stirring at 120^0 C on sand bath for 8h. The progress of reaction was checked by TLC. After completion of reaction ice cold water was added and the mixture was extracted with chloroform three times. The pooled chloroform extract was washed with 7% aquous solution until it was neutralized and dried under reduced pressure. The chloroform extract was purified by column chromatography using silica gel as adsorbent and hexane :ethyl acetate (95:5) as eluent, which resulted the white crystal (150mg)with 75% yield

^{1}H NMR ($CDCl_3$, 300 MHz): (0.88 (3H, t, H-14'), 1.26 (10 x 2H, br s, H-4'-13'), 1.74 (2H, m, H-3), 2.56 (2H, t, H-2'), 3.42 (2H, d, H-8), 3.80 (3H, s, H-7 -OCH3), 5.10 (2H, t, H-10), 5.95 (1H, m, H-9), 6.74 (1H, s, H-3), 6.77 (1H, d, H-5), 6.91 (1H, d, H-6). ^{13}C NMR ($CDCl_3$, 75.5 MHz): 138.8s (C-1), 151.5s (C-2), 113.3d (C-3), 139.2s (C-4), 122.9d (C-5), 121.6d (C-6), 56.2q (C-7), 40.4t (C-8), 137.5d (C-9), 116.4t (C-10), 172.8s (C-1'), 34.4t (C-2'), 25.4t (C-3'), 29.4t (C-4'), 29.6-29.9t (C-5'-10'), 30.0t (C-11'), 32.2t (C-12'), 22.9t (C-13'), 14.3q (C-14').

Eugenyl laurate (104)

To a solution of eugenol (200mg) in pyridine (5ml), palmitoyl chloride (177 mg) was added .The airtight reaction mixture was continued to stirring at 120^0 on sand bath for 7h. The progress of reaction was checked by TLC. After completion of reaction ice cold water was added and the mixture was extracted with chloroform three times. The pooled chloroform extract was washed with 7% aquous solution until it was neutralized and dried under reduced pressure . The chloroform extract was purified by column chromatography using silica gel as adsorbent and hexane :ethyl acetate (97:3) as eluent, which resulted the white shiny crystal (160mg)with 80% yield. ^{1}H NMR ($CDCl_3$, 300 MHz): (0.88 (3H, t, H-12'), 1.27 (16H, br s, H-4'-11'), 1.75 (2H, m, H-3'), 2.54 (2H, t, H-2'), 3.37 (2H, d, H-8), 3.8 (3H, br s, H-7), 5.92 (1H,m, H-9), 5.10 (2H, t, H-10), 6.74 (1H, s, H-3), 6.77 (1H, d, H-5), 6.94 (1H, d, H-6). ^{13}C NMR ($CDCl_3$, 300 MHz): 138.8s (C-1), 151.5s (C-2), 116.3d (C-3), 139.1s (C-4), 121.1d (C-5), 122.9d (C-6), 56.2q (C-7), 40.3t (C-8), 137.5d (C-9), 113.4t (C-10), 172.2s (C-1'), 34.4t (C-2'), 25.4t (C-3'), 29.4t (C-4'), 29.6-29.9t (C-5'-9'), 32.2t (C-10'), 22.9t (C-11'), 14.4q (C-12').

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