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Isolation and characterization of substituted dibutyl phthalate from *Ipomoea carnea* stem

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ABSTRACT

Medicinally important plant species Ipomoea is reported in Indian system of medicine from ancient times. Ipomoea carnea is one of those reported as used in folk medicine. It produces a variety of secondary metabolites. Chemical investigation by chromatographic techniques was employed. Bioactive ethyl acetate extract of I. carnea stem bark was used for separation of the components. Substituted dibutyl phthalate was isolated for the first time from this plant source. The structure of the isolated compound was elucidated by IR, MS, ^{1}H-NMR, ^{13}C- NMR, DEPT, HMBC and HSQC. The compound was identified as Butyl- (E)- 3' hydroxyl- 7',7'- dimethyl - oct- 5'- enyl –phthalate.

Key words: *Ipomoea carnea*, extract, bioactive, substituted dibutyl phthalate.

INTRODUCTION

Ipomoea carnea is a native of South America and available plenty in all the states of India due to its adaptation to the Indian climatic conditions [1]. It belongs to convolvulaceae family and fistulosa subfamily [2]. Several reports are available on the biological activities of *I. carnea*. It is reported for wound healing activity [3]. Extract of the whole plant was widely used as antirheumatic remedy in Bolivia [4]. Immunomodulatory activity of aqueous extract of *I. carnea* was tested on peritoneal cells of rat [5]. Effects of alkaloids from *I. carnea* on intracellular lysosomal glycosidase activity in humane lymphoblast culture were studied [6]. The clinical, biochemical, hematological and pathological effects of long term administration of *I. carnea* to growing goats were reported [7]. Published work was available on the effect of aqueous extract of *I. carnea* leaf on isolated frog and mouse hearts [8]. Antimicrobial activity of metal complexes prepared from the leaves proteins of *I. carnea* was reported [9]. There are reports on synergistic effect of insecticides with plant extracts of *I. carnea* against malarial vector, *Anopheles stephensi*[10]. Aqueous and petroleum ether extracts of *I. carnea* leaves have the potential to be used as an ideal eco-friendly approach for the control of the major lymphatic filariasis vector, *Culex quinquefasciatus* [11]. A saponin had been isolated from *I. carnea* with anticarcinogenic and oxytoxic properties [12]. Chouwdhury *et al*. reported its other bio activity [4].

In a continuing research of isolation of bioactive metabolites from plants, a secondary metabolite, substituted dibutyl phthalate had been isolated and reported for the first time from *I. carnea* stem bark. Isolation, characterization and activity study of dibutyl phthalate from *Ipomoea carnea* has been reported by author's [13,14]. Dibutyl phthalate had been also isolated from, marine algae, bacteria, fungi[15] and from *Mimusops elengi*[16], *Leea indica*[17], *Alstonia scholaris, Torreya grandis, Achyrathes bidentata* & *Rheum glabricaule*[18] .

The structure of the isolated compound was elucidated by IR, MS, ^{1}H-NMR, ^{13}C- NMR, HMBC and HSQC etc.

MATERIALS AND METHODS

All the reagents used were products of Merck, all AR grade. The IR spectrum (4000 -350 cm^{-1}) was recorded on Shimadzu FTIR-84005 spectrophotometer. NMR spectrum was recorded on Bruker Armce III (400 MHz) machine. Mass spectrum was recorded on Sciex API 3000 (ESI) spectrometer.

Collection and identification of plant materials

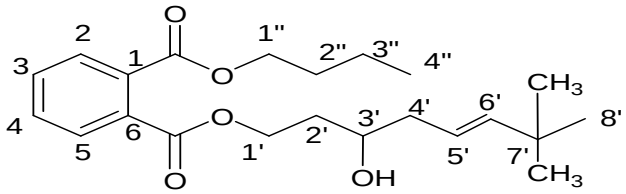
The plant material was collected from the river sides of Pune, Maharashtra, India. The plant was authenticated at Botanical Survey of India, Pune, India. Its authentication no is ELICAI.,BSI/WC/Tech/2009/96.

Preparation of plant extract and isolation

Air shade dried and pulverized stem material of *I. carnea* was refluxed with ethyl acetate for eighteen hours. Solvent was recovered under reduced pressure to obtain the crude mass (**EA**, 7%.). Broad fractionation of **EA**, was carried out with gradient polarity of solvents using silica gel (60- 120, 10 g) to get nine major fractions as (**A**, hexane), (**B**, hexane :ethyl acetate, 9.5:0.5), (**C**,hexane: ethyl acetate 9: 1), (**D**, hexane: ethyl acetate, 8:2), (**E**, hexane: ethyl acetate,1:1), (**F**, ethyl acetate), (**G**, acetone), (**H**, ethanol) and (**I**, methanol). Fractions were monitored by thin layer chromatography. Details are reported (**Table 1)**.

Fraction **B** (**Table 1**) was a mixture of unidentified compounds along with compound **1**. Re-chromatography of fraction **B** was performed using gradient polarity of solvents on silica gel and total eight fractions (**A'** to **H'**) were collected (**Table 2**). Fraction **B'** was an impure solid**,** compound **1.** It was purified by repeated crystallization using ether- hexane as mixed solvent system which furnished a white amorphous solid - **compound 1.**

RESULTS AND DISCUSSION



Butyl- (E)- 3' hydroxyl- 7',7'- dimethyl - oct- 5'- enyl –phthalate

The compound **1** is a white amorphous solid. It shows sharp melting nature at 180^{o} C.

The mass spectrum (Fig. 1) of the compound exhibits a molecular ion peak at *m/z* 376 and a base peak at *m/z* 278 which is in accordance with the molecular formula **$C_{22}H_{32}O_5$**.

IR spectrum, Fig.2 (KBr, *v*, cm^{-1}): 3439 (hydroxy group); 1707 (ester carbonyl); 1287, 1233 and 1190 (-C-O- stretching) ; 725 (ortho disubstitution).

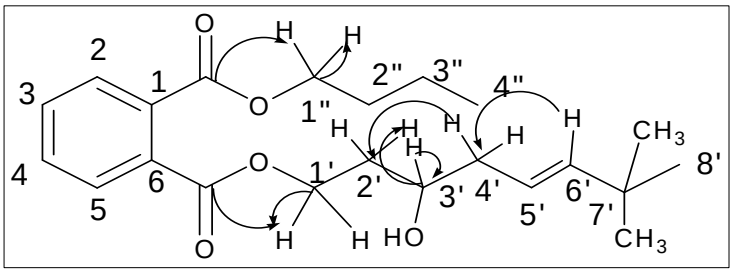
PMR spectrum, Fig. 3 (500 MHz, $CDCl_3$, δ ppm, *J*/Hz); 7.72 (2 H, dd, *J* =9.2 & 2.4 *Hz*, H 2 & H 5) ; 7.53 (2 H, dd, *J* = 5.6 & 2.4 *Hz*, H 3 & H 4);5.32 (1H, m, H5') ; 5.30 (1H, m, H6') 4.30 (2H, t, H 1') ; 4.10 (2H, t, H 1") ; 4.15 (1H, m, H3'); 3.64 (hydroxyl proton). 2.31(2H, m, H 4') ; 2.04 (2H, m, H2') ;1.53 (4H, m, H2" & H3") . 0.89 (12H, CH_3-7', CH_3- 8' and CH_3- 4").

^{13}C NMR spectrum, Fig. 4 (125 MHz , $CDCl_3$)

172.24 (ester carbonyl carbon);132 (s, C-1& C-6) ; 31.10 (s, C-7'); 144.00 (d, C-6') ; 122.77(d, C-5') ;129.87 (d, C-2 & C-5) ; 127.81 (d, C-3 & C-4); 68 (d, C -3'); 62.00 (t, C1' and C1") ; 34.69 - δ 21.67 (t, C2', C4', C2" and C3" as a methylene envelop) ; 31.10, 18.80 , 13.70 (q, methyl groups attached to C7' and C4") .

From the heteronuclear single quantum coorelation, Fig. 5 (**HSQC**) spectrum, it is marked that downfield shift of aromatic protons at δ 7.72 and δ 7.53 coincide with aromatic carbon atoms (C2 & C5, δ129.87) and (C3& C4,

δ127.81). The relationship of olifinic protons H5' & H6' (δ 5.32 and δ 5.30) is noticed with C5' and C6' (δ122.77 & δ 144.00). The coexistence of H1' & H1" at δ 4.30 and 4.10 is in agreement with C1' and C1" at δ 62.00. The methylene protons H 2' and H 4' at δ 2.04 and δ 2.31 establish correlation with C2' and C4' respectively. The methyl protons at δ 0.89 ascertain coorelation with methyl groups attached to C7' and C 4" (δ 31.10, δ18.80 and δ13.70). The ^{1}H NMR assignments are confirmed by the ^{2}J CH and ^{3}J CH correlations in the heteronulear multiple bond correlation, Fig. 6 (**HMBC**) spectrum. The absence of homonulear coupling at δ132 and δ 31.10 for (C1& C6) and C7' indicates totally substituted carbon atoms. In HMBC spectrum the ester carbonyls at δ172.24, being equivalent in nature, show ^{3}J CH correlations with H1' and H1" at δ 4.30 and δ 4.10. The methine proton H 3' at δ 4.15 shows coupling with C3' carbon atom. Methine carbon C 3' at δ 68 shows connectivity to H2' proton at δ 2.04. The correlation of C1' and C1" at δ 62 show connectivity with H1' and H1" at δ 4.30 and δ 4.10 protons respectively. The correlation of H6' at δ 5.3 shows connectivity with C4' at δ 27.96 of methylene envelops. The correlation of H 4' at δ 2.31 shows connectivity with C2' at δ 29.24 of methylene envelops. Correlation experiments (HMBC) confirmes the position of double bonds and hydroxy function.The connectivity of acetoxy carbonyl carbons to oxy methylenes confirms their positions.



HMBC correlation of compound 1

Table 1 Chromatographic separation of ethyl acetate (EA) extract

Fr.	Eluent	Volume (ml)	Weight (g)	Approximate composition
A	Hexane	6 x100	1.645	Mixture of unidentified compounds
B	hexane:ethyl acetate(9.5:0.5),	5 x100	0.383	Mixture of unidentified compounds + **Compound 1**
C	hexane:ethyl acetate(9: 1)	5 x100	0.209	Mixture of unidentified compounds
D	hexane: ethyl acetate (8:2)	5 x100	0.190	Mixture of unidentified compounds
E	hexane: ethyl acetate (1:1)	5 x100	0.500	Mixture of unidentified compounds
F	Ethyl acetate	6 x100	0.300	Mixture of unidentified compounds
G	Acetone	5 x100	0.221	Mixture of unidentified compounds
H	Ethanol	5 x100	0.300	Mixture of unidentifieds compounds
I	Methanol	5 x100	0.250	Mixture of unidentified compounds

Table 2 Rechromatograghy of Fraction B

Fraction	Eluent	Volume (ml)	Weight (g)	Approximate composition
A'	Hexane	10 x100	0.300	Mixture of unidentified compounds
B'	Hexane:Ethyl acetate(9.5:0.5)	10 x100	0.500	Mixture of unidentified compounds + **Compound 1**
C'	Hexane:Ethyl acetate(9: 1)	8 x100	0.100	Mixture of unidentified compounds
D'	Hexane:Ethyl acetate (8:2	8 x100	0.120	Mixture of unidentified compounds
E'	Hexane:Ethyl acetate (1:1)	8 x100	0.105	Mixture of unidentified compounds
F'	Ethylacetate	6 x100	0.090	Mixture of unidentified compounds
G'	Acetone	6 x100	0.050	Mixture of unidentified compounds
H'	Methanol	4 x100	0.040	Mixture of unidentified compounds

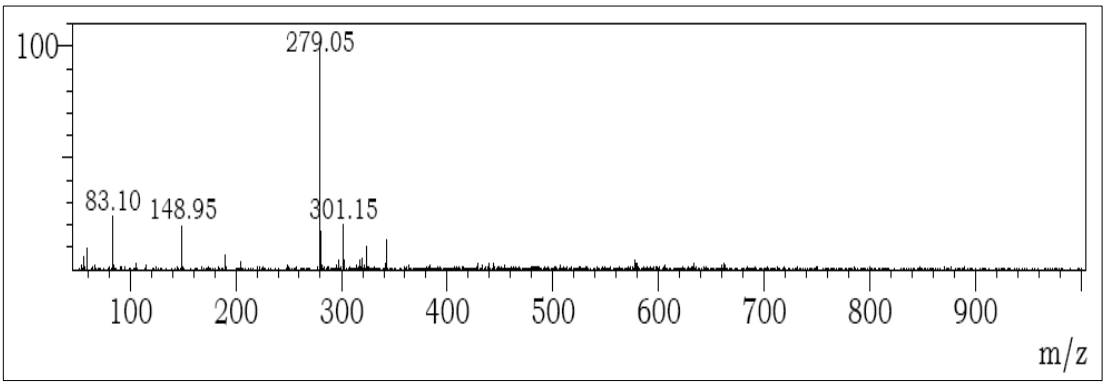


Fig. 1

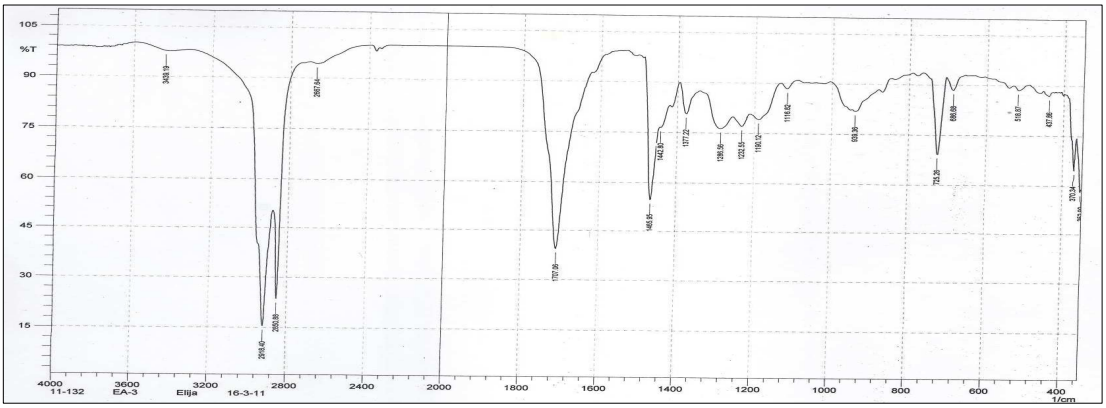


Fig. 2

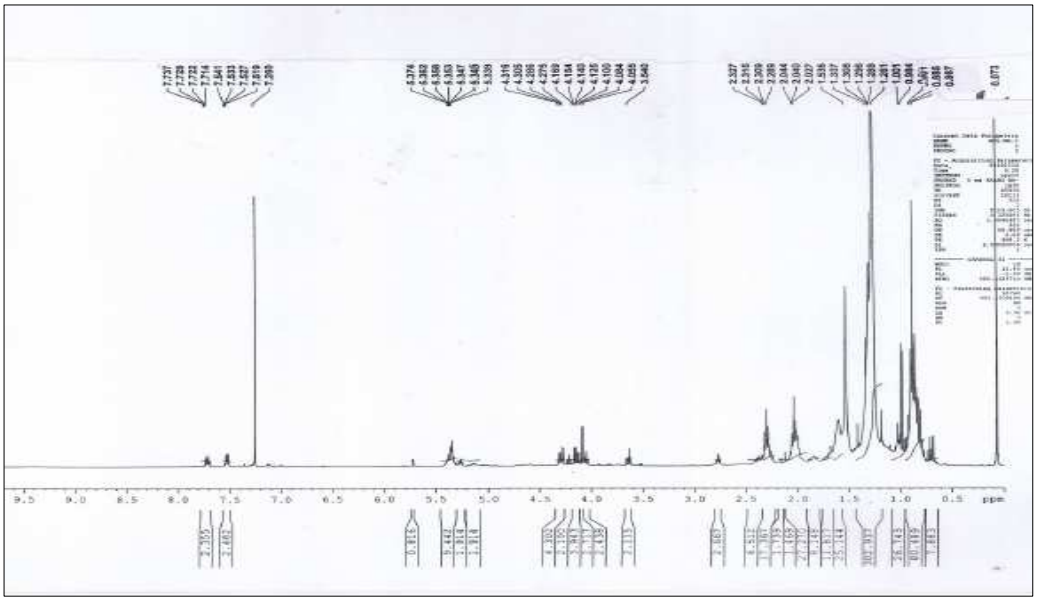


Fig. 3

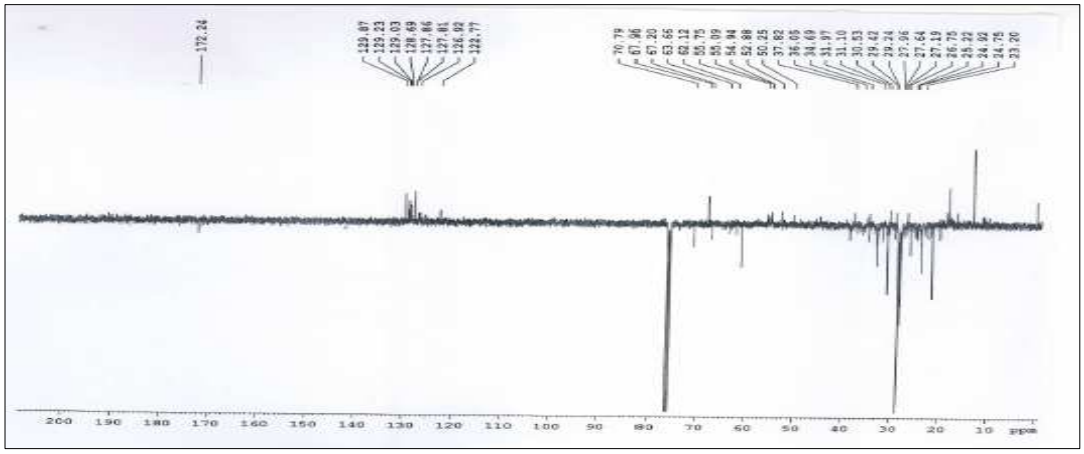


Fig. 4

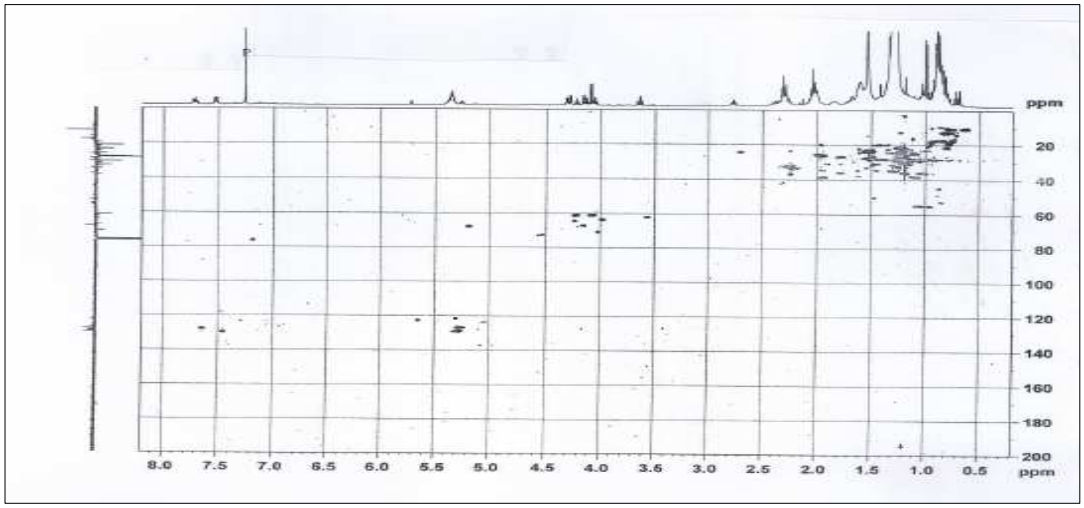


Fig. 5

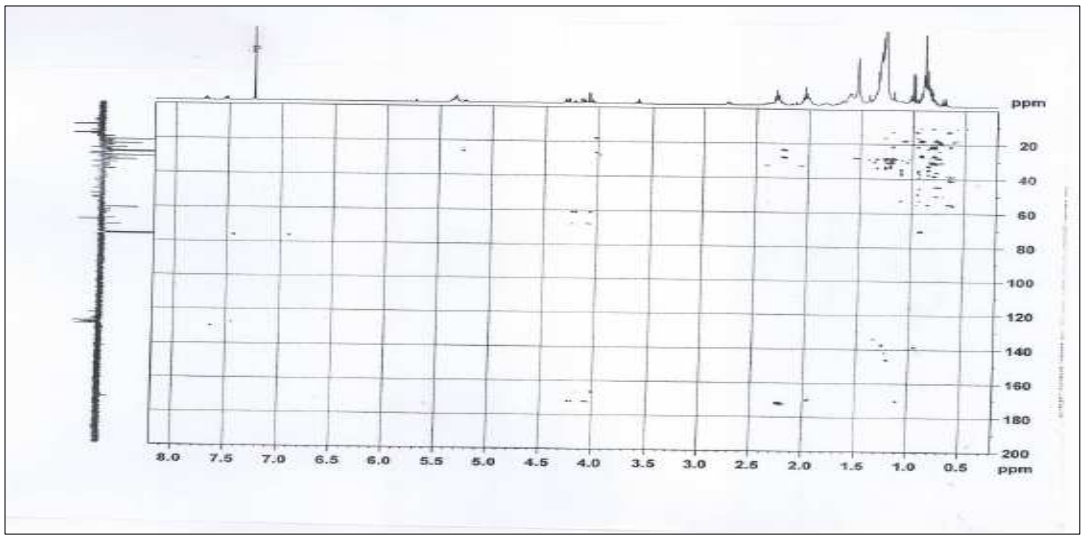


Fig.6

CONCLUSION

The isolated compound is a derive condensed product of dibutyl phthalate. It is isolated for the first time from the plant source. Since dibutyl phthalate was found to possesses antimicrobial as well as potent larvicidal activity, so its derivative, butyl- (E)- 3' hydroxyl- 7',7'- dimethyl - oct- 5'- enyl –phthalate might be bioactive.

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