



TERMINALIA TOMENTOSA ROXB (ex DC) WIGHT & ARN: PHYTOCHEMICAL INVESTIGATION

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ABSTRACT

The present study was undertaken to carry out phytochemical investigation of the ethanolic extract of the stem bark of Terminalia tomentosa Roxb (ex DC) Wight & Arn belonging to the family Combretaceae. The plant is known in Sanskrit as Asana, in English as Black murdah, in Hindi as Asan, Saj, Sain and in Marathi as Ain. The plant has been known to possess various pharmacological activities like antifungal, antioxidant, antihyperglycaemic, antidiarrhoeal, antileucorrhoeal etc. The bark of the plant is astringent & useful in ulcers, vata, fractures, haemorrhages, bronchitis, diarrhoea etc. Preliminary phytochemical screening of the ethanolic extract of stem bark revealed the presence of carbohydrates, flavonoids, triterpenoids, steroids, tannins and saponins. The chemical entities isolated and characterised includes 4 - methyl - 4 - hydroxymethylene - 6 β - (10 - methyl octanyl) cyclohexane (Arjuna homoses quiterpenol), di-n-octyl phthalate, di isobutyl phthalate and dibutyl phthalate. All these phytoconstituents are reported for the first time from the ethanolic extract of stem bark of T. tomentosa.

Keywords: Terminalia tomentosa, Combretaceae, Di-n-octyl phthalate, Triterpenoids.

INTRODUCTION

Terminalia tomentosa Roxb (ex DC) Wight & Arn (Synonyms: *Terminalia alata* Heyne ex Roth, *Terminalia crenulata* Roth, *Terminalia elliptica* Willd.) is a large deciduous tree, 20-35m high & 1m in diameter belonging to family Combretaceae^{1,2,3,4}. The bark is rough, dark

grey to black in colour with deep vertical fissures & transverse cracks^[5]. Leaves are simple, sub-opposite or the uppermost alternate, thick coriaceous, ovate-oblong or elliptic-oblong, rarely obovate, softly tomentose when young; becoming more or less glabrous when mature, with 1-2 glands (

which are often turbinate or long stalked) usually on the midrib but sometimes absent. Flowers are hermaphrodite and in axillary fulvous-pubescent spikes or terminal panicles. Fruits are 1¹/₂ -2 inches long and 3/4 inch wide with 5 broad, coriaceous, brown, glabrous wings striated with numerous straight lines running horizontally from the axis to the edges^[6]. The plant is common in the forests, especially in the humid regions of India, including the sub-Himalayan tracts of North West provinces, Nepal & Sikkim, also Southwards throughout the Peninsula ⁷. It is a prominent part of both dry and moist deciduous forests in southern India up to 1000 m. The bark is bitter & styptic, useful in vitiated conditions of pitta, ulcers, vata, fractures, haemorrhages, bronchitis cardiopathy, strangury, wounds, haemoptysis, dysentery, cough, verminosis ,leucorrhoea, gonorrhoea & burning sensation (Ayurveda)^[8,9]. Phytoconstituents such as tannins like arjunic acid, arjunolic acid, arjunetin, ellagic acid, gallic acid, and triterpenoids like oleanolic acid, betulinic acid and steroid like β -sitosterol have been reported to be present in *T.tomentosa*^{10, 11, 12, 13, 14}. The plant is known to possess many pharmacological properties like antifungal¹⁵, antioxidant^[16], anti-hyperglycaemic¹⁷, anti-diarrhoeal & anti leucorrhoeal¹⁸. From the literature survey, it was learnt that no substantial work has been carried out on the stem bark of *T.tomentosa*. Hence an attempt was made to investigate the phytoconstituents from ethanolic extract of *T.tomentosa* stem bark.

MATERIALS AND METHODS

All the melting points were recorded in Bio Technics India, Model no.BT2-38 melting point apparatus & were uncorrected. IR spectra of the compounds were recorded using the KBr pellet method on a Bruker α -T Spectrophotometer, at National facility for Clinical Trial, ISISM Chennai.¹HNMR &

LC-MS spectra of compounds were taken on Bruker 500 MHz PMR Spectrophotometer using CDCL₃ as solvent & Shimadzu LC 2020 at National facility for Clinical Trial, ISISM Chennai. ESI-MS spectra were recorded using ESI-MS Expression CMS Advion at SynZeal Research Laboratory, Ahmedabad. TLC was carried out using Aluchrosep Silica gel 60/UV254 from S.D. Fine Chemicals Pvt. Ltd, Mumbai. Column chromatography was carried out using glass column with a glass stop cock, 30×600mm from Merck Specialities Pvt. Ltd, Mumbai, packed with silica gel (200-400 mesh) from Molychem, Mumbai. All the chemicals & reagents used were obtained in high purity from S.D. Fine chemicals Pvt. Ltd, Bombay, Molychem & Chemport Pvt. Ltd, Mumbai.

Plant Material⁶

The stem bark of *T. tomentosa* was collected from Darbandora, Ponda- Goa during October 2012. It was authenticated by Prof G. I. Hukkeri, Dept. of Botany, Dhempe College of Arts & Science, Miramar-Goa.

The stem bark was then washed thoroughly to remove the soil and adhering materials and dried in shade. The dried stem bark was powdered and used for the preparation of ethanolic extract.

Preparation of ethanolic extract¹⁹

The dried stem bark powder (500g) was extracted by maceration with ethanol (95%) for 3 days. After 3 days ethanolic layer was decanted off. The process was repeated thrice. The solvent from the total extract was distilled off using Rotary vacuum evaporator (Superfit) and the concentrate was evaporated to a syrupy consistency and then evaporated to dryness (80g)

Preliminary Phytochemical Screening (Qualitative Analysis)^{20,21}

The preliminary phytochemical studies were performed for testing the different phytoconstituents present in the ethanolic extract of the stem bark of *T.tomentosa* per the standard procedures. The results are tabulated in table 1.

Isolation of Compounds from Ethanol Soluble Fraction¹⁹

The ethanol soluble fraction (10g) was mixed with silica gel (2g). The sample was then loaded on column previously packed with 150g of Silica gel (Molychem, 200-400 mesh) prepared in petroleum ether (60-80°C). The column was subjected to different solvent systems, starting first with petroleum ether 100% followed by petroleum ether:chloroform graded mixtures (95:5, 90:10, 80:20, 70:30, 60:40, 50:50) then with chloroform 100% followed by graded mixtures of chloroform : ethyl acetate (95:5, 90:10, 80:20, 70:30, 60:40, 50:50) & finally with ethyl acetate 100% & graded mixtures of ethyl acetate: methanol (99:1, 98:2, 97:3, 96:4, 95:5). The elutions were monitored by TLC (Silica gel-G), visualization by UV 254, 366nm & vanillin-sulphuric acid spraying reagent heated at 110°C. Each time 10ml elutes were collected & identical elutes were combined (TLC monitored) & concentrated to 5ml & kept aside.

Elutions carried out with graded mixture of petroleum ether (60-80°C): (80:20) chloroform, resulted a single component on TLC (petroleum ether (60-80°C): chloroform, 80:20). After removal of the solvent, an off white powder was obtained, which was designated as **Compound AM 1** (70mg).

Elutions carried out with ethyl acetate:methanol (99:1) resulted into a single component on TLC (ethyl acetate:methanol, 99:1). After removing

solvent yellow liquid resulted, this was designated as **Compound AM 2** (62mg).

Elutions carried out with ethyl acetate:methanol (97:3) resulted into a single component on TLC (ethyl acetate:methanol, 97:3). After removing solvent yellow viscous liquid resulted, this was designated as **Compound AM 3** (55 mg).

Elutions carried out with ethyl acetate:methanol (95:5) resulted into a single component on TLC (ethyl acetate:methanol, 95:5). After removing solvent pale yellow viscous liquid resulted, this was designated as **Compound AM 4** (45 mg).

Elutions carried out with other graded mixtures of solvents resulted in resinous mass which was not processed further.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the ethanolic extract of stem bark of *T.tomentosa* was performed and it was found to contain carbohydrates, flavonoids, triterpenoids, steroids, tannins and saponins. The chemical investigation led to the isolation of four compounds from the ethanolic extract of the stem bark of *T.tomentosa*. The isolated compounds were 4 - methyl - 4 - hydroxymethylene - 6 β - (10 - methyl octanyl) cyclohexane (Arjunahomosesquiterpenol), di-n-octyl phthalate, di isobutyl phthalate and dibutyl phthalate.

Compound 1 (Arjuna homosesquiterpenol): m.p. 152°C; IR (KBr): 3437.39 cm^{-1} (br, OH), 2925.14 cm^{-1} (C-H str. in CH₃), 2858.08 cm^{-1} (C-H str. in CH₂), 1458.11 cm^{-1} (C-H deformation in CH₃), 1279.29 cm^{-1} (C-O str.); ¹H NMR (CDCl₃): δ 0.779 - δ 0.956 (m, 3H, H-14), δ 0.977 (s, 3H, H-17), δ 1.078 (brs, 3H, H-16), δ 1.258 (s, 12H, 6xCH₂), δ 1.511 - δ 1.550 (m, 4H, H-2, H-5 of the ring), δ 1.557

(s,4H, H-1, H-3 of the ring), δ 2.314- δ 2.393 (m,1H, H-10 α), δ 2.413- δ 2.592 (m, 1H, H-6 α), δ 3.730- δ 3.815 (m,1H, H2-15b), δ 3.828 (s,1H,OH), δ 4.035- δ 4.089 (m,1H,H2-15a); In the LCMS spectrum of Arjuna homoses quiterpenol, it exhibited molecular ion peak at m/z 255.10 $[M+H]^+$ which was consistent with molecular formula of C₁₇H₃₄O. Fragment ion peaks at m/z 239.20 $[M-Me]^+$, 225.10 $[M-C_2H_5]^+$, 223.25 $[M-CH_2OH]^+$, 211.45 $[M-C_3H_7]^+$, 197.10 $[M-C_4H_7]^+$, 169.45 $[M-C_6H_{13}]^+$, 155.30 $[M-C_7H_{15}]^+$, 141.25 $[M-C_8H_{17}]^+$, 127.25 $[M-C_9H_{19}]^+$ suggested that the molecule possessed a C₉-side chain attached to a hydroxyl substituted dimethyl cyclohexane ring²². (Figure 1).

Compound 2 (Di-n-octyl phthalate): b.p. 3790C; IR (KBr): 2927.17 cm^{-1} (C-H str. in CH₃), 2860.76 cm^{-1} (C-H str. in CH₂), 1727.64 cm^{-1} (C=O str.), 1628.74 cm^{-1} (C=C str.), 1459.24 cm^{-1} (C-H deformation in CH₃), 744.07 cm^{-1} (C-H bending of aromatic ring); ¹HNMR (CDCl₃): δ 0.866- δ 0.997 (m, 10H, H-7', 7'', 8', 8''), δ 1.364- δ 1.439 (m, 12H, H-4', 4'', 5', 5'', 6', 6''), δ 1.674- δ 1.747 (m, 8H, H-2', 2'', 3', 3''), δ 4.184- δ 4.256 (m, 4H, H-1', 1''), δ 7.510- δ 7.535 (m, 2H, H-4,5), δ 7.697- δ 7.723 (m, 2H, H-3,6); The ESI-MS spectrum showed molecular ion peak at m/z 391.4 $[M+H]^+$ in the positive ion mode which was consistent with molecular formula of C₂₄H₃₈O₄. Fragment ion peaks at m/z 279.2, m/z 167.1 exhibited in the mass spectrum were characteristic of alkyl phthalates & the base peak at m/z 149 is due to the protonated phthalic anhydride (C₈H₅O₃)²³. (Figure 2).

Compound 3 (Di isobutyl phthalate): b.p. 319°C; IR (KBr): 2928.50 cm^{-1} (C-H str. in CH₃), 2861.96 cm^{-1} (C-H str. in CH₂), 1727.99 cm^{-1} (C=O str.), 1603.24 cm^{-1} (C=C str.), 1457.21 cm^{-1} (C-H deformation in CH₃), 1279.29 cm^{-1} (C-O str.), 1125.66 cm^{-1} (C-H deformation in CH₂), 830.36 cm^{-1} (C-H

bending of aromatic ring); ¹HNMR (CDCl₃): δ 0.863- δ 1.684 (m, 12H, H-3', 3'', 4', 4''), δ 3.574- δ 3.621 (m, 2H, H-2', 2''), δ 4.202- δ 4.319 (m, 4H, H-1', 1''), δ 7.513- δ 7.537 (m, 2H, H-3,4), δ 7.693- δ 7.723 (m, 2H, H-2,5); The ESI-MS spectra showed molecular ion peak at m/z 278.3 $[M]^+$ which was consistent with molecular formula of C₁₆H₂₂O₄. The mass spectrum showed fragment ion peaks at m/z 167.1 & m/z 149 which are considered characteristic of alkyl phthalates & m/z 223.2 suggested that the alkyl phthalate is Di isobutyl phthalate. The ion peak at m/z 149 is due to the protonated phthalic anhydride (C₈H₅O₃)²⁴. (Figure 3).

Compound 4 (Dibutyl phthalate): b.p. 337°C; IR (KBr): 2925.79 cm^{-1} (C-H str. in CH₃), 2858.25 cm^{-1} (C-H str. in CH₂), 1728.23 cm^{-1} (C=O str.), 1637.65 cm^{-1} (C=C str.), 1458.18 cm^{-1} (C-H deformation in CH₃), 1073.39 cm^{-1} (C-O str.), 742.94 cm^{-1} (C-H bending of aromatic ring); ¹HNMR (CDCl₃): δ 0.896- δ 0.998 (m, 6H, H-4', 4''), δ 1.257- δ 1.424 (m, 4H, H-3', 3''), δ 1.719 (s, 4H, H-2', 2''), δ 4.184- δ 4.320 (m, 4H, H-1', 1''), δ 7.505- δ 7.543 (m, 2H, H-4,5), δ 7.689- δ 7.723 (m, 2H, H-3,6); The ESI-MS spectrum showed molecular ion peak at m/z 279.2 $[M+H]^+$ which was consistent with molecular formula C₁₆H₂₂O₄. The mass spectrum showed fragment ion peaks at m/z 167.1 & m/z 149 which are considered characteristic of alkyl phthalates & used in their characterization. The fragment ions at m/z 205.4 & 223.2 suggested that the alkyl phthalate is Dibutyl phthalate. The base peak at m/z 149 is due to the protonated phthalic anhydride (C₈H₅O₃)²⁴. (Figure 4).

CONCLUSION

The chemical investigation led to the isolation of four compounds from the ethanolic extract of the stem bark of *T. tomentosa*, which includes 4-methyl - 4 - hydroxymethylene - 6 β - (10 -methyl octanyl) cyclohexane (Arjunahomosesquiterpenol), di-

n-octyl phthalate, di isobutyl phthalate and dibutyl phthalate. The isolated and characterised constituents can be categorised under the class of sesquiterpenoids and phthalate derivatives. The above compounds were isolated for the first time from the ethanolic extract of stem bark of *T. tomentosa*.

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Table 1. Result of Qualitative analysis for phyto-constituents isolated from the ethanolic extract of the stem bark of *T.tomentosa* W& A.

SR.NO	CHEMICAL TEST	INFERENCE
1.	Alkaloids	-
2.	Carbohydrates	+
3.	Flavonoids	+
4.	Triterpenoids	+
5.	Steroids	+
6.	Tannins	+
7.	Resins	-
8.	Proteins	-
9.	Glycosides	-
10.	Starch	-

Present = + Absent = -

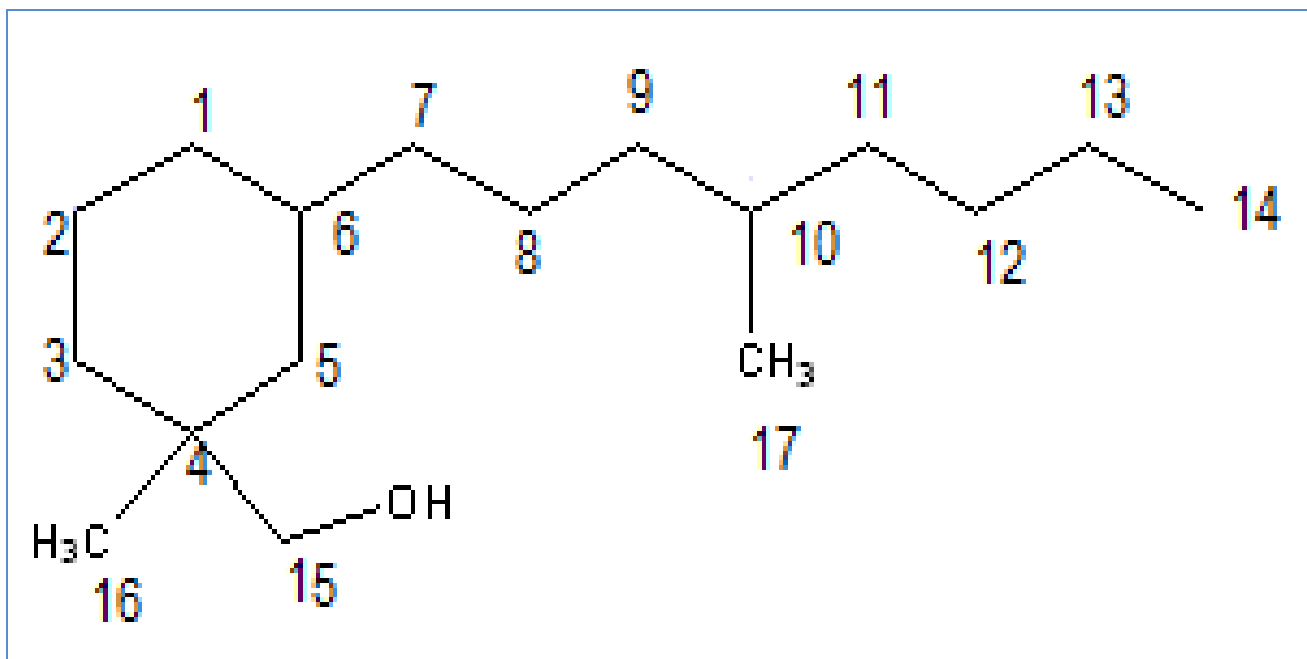


Figure.1. 4-methyl-4-hydroxymethylene-6β-(10-methyl octanyl) cyclohexane (Arjuna homosesquiterpenol)

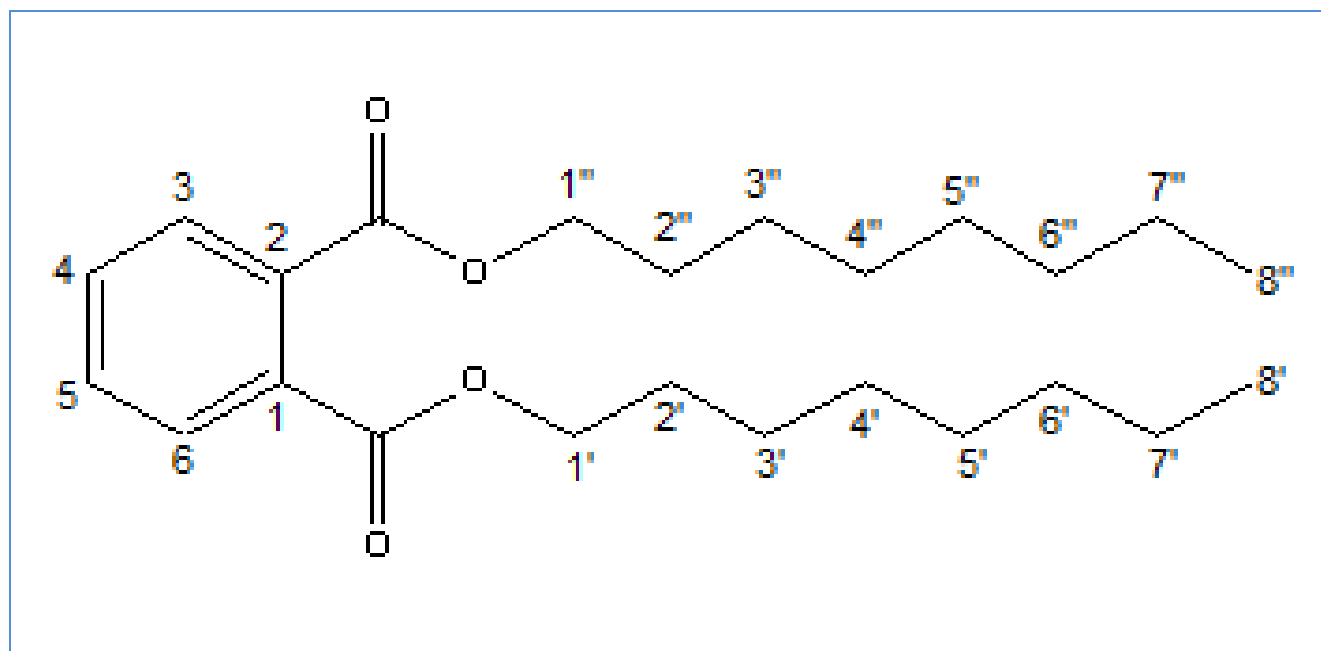


Figure.2. Di-n-octyl phthalate

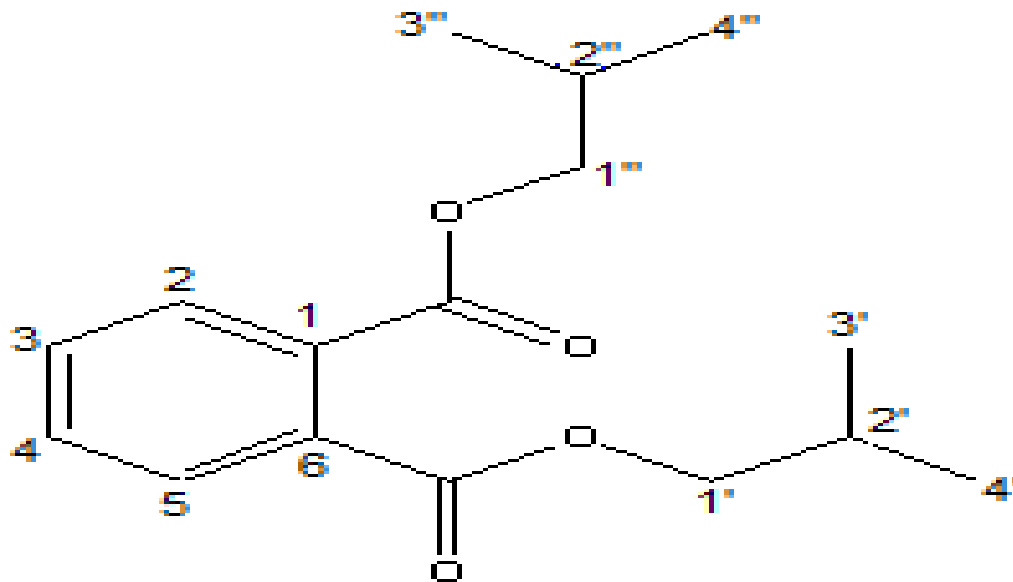


Figure.3. Di isobutyl phthalate

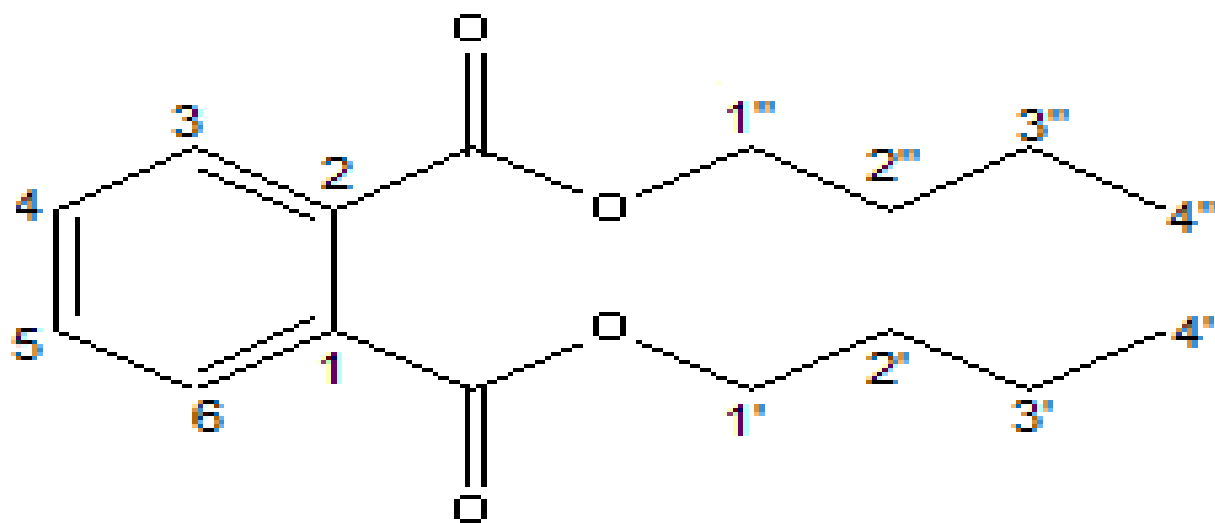


Figure.4. Dibutyl phthalate