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## Isolation of Lupeol, Stigmasterol and Campesterol from Petroleum Ether Extract of Woody Stem of *Wrightia tinctoria*

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**Abstract:** Aim of this study was to identify and characterize the bioactive principles from the woody stem of *Wrightia tinctoria*. For isolation of the compounds, the dried woody stem's powder of *Wrightia tinctoria* was subjected to hot extraction with petroleum ether and subjected to chromatography. Three compounds (PEW-1, PEW-2 and PEW-3) were isolated and purified by chloroform. Mass spectrum of PEW-1, PEW-2 and PEW-3 showed a parent molecular ion [M<sup>+</sup>] peak at mlz 426 which corresponds to the molecular formula C<sub>30</sub>H<sub>50</sub>O, 412 corresponds to C<sub>29</sub>H<sub>48</sub>O and 400 corresponds to C<sub>28</sub>H<sub>46</sub>O. In the <sup>1</sup>H-NMR spectrum of PEW-1, H-3 proton appeared as a triplet of a double doublet (tdd) at δ 3.21, H-29 proton gives two multiplets at δ 4.71 and δ 4.56, in <sup>1</sup>H-NMR spectrum of PEW-2, H-3 proton appeared as a triplet of a double doublet (tdd) at δ 3.62 and H-6 olefinic proton showed a multiplet at δ 5.14. Two olefinic protons appeared downfield at δ 4.16 (m) and δ 4.14 (m). Six methyl protons appeared at δ 1.27, δ 1.19, δ 1.07, δ 1.00, δ 0.98 and δ 0.91 for methyl group and in the <sup>1</sup>H-NMR data of PEW-3, H-3 proton appeared at δ 3.21 as a triplet of a double doublet H-6 olefinic proton showed a multiplet at δ 5.10 and Six methyl protons appeared at δ 1.27, δ 1.14, δ 1.09, δ 1.00, δ 0.98 and δ 0.95 for methyl group. From the physical, chemical and spectral characteristics, PEW-1, PEW-2 and PEW-3 were concluded as lupeol, stigmasterol and campesterol.

**Key words:** *Wrightia tinctoria*, apocynaceae, petroleum ether

### INTRODUCTION

The use of higher plants and their preparations to treat infectious diseases is an age-old practice and in the past possibly the only method available. However, the systematic study of higher plants for detecting antimicrobial activity is of comparatively recent origin (Peach and Tracey, 1955). These investigations have been triggered by the emergence and spread of antibiotic resistant microorganisms causing the effective life-span of existing antibiotics limited (Cowan, 1999). Hence, the plant kingdom is being screened for newer and effective chemotherapeutic agents. Higher plants can serve both as potential antimicrobial crude drugs as well as a source of new anti-infective agents.

*Wrightia tinctoria* (Roxb.) R. Br. Belongs to family Apocynaceae (Anonymous, 1976) the wealth of India), is a small deciduous tree, generally up to 1.8 m tall and often under 60 cm girth, sometimes up to 7.5 m high, distributed all over India (Kiritkar and Basu, 1994). Four uncommon sterols, desmosterol, clerosterol, 24-methylene-25-methylcholesterol and 24-dehydropollinastanol, in

addition to several usual phytosterols, were also isolated and identified (Akihisa *et al.*, 1988). The wrightial, a new terpene and other phytoconstituents such as cycloartenone, cycloeucalenol were isolated identified by fractionation of methanol extract of the immature seed pods (Ramchandra *et al.*, 1993). The five flavonoid compounds, Indigotin, Indirubin, tryptanthrin, isatin and rutin were isolated and identified from the leaves (Muruganadam *et al.*, 2000). The unsaponifiable matter extracted from bark by petroleum ether extract was fractionated with methanol to yield a triterpenes like β-sitosterol and β-amyrin, wrightadiene (Warrier *et al.*, 1996). A stable serine protease, wrightin has been isolated from the latex of the plant *Wrightia tinctoria* (Tomar *et al.*, 2008). The characterization of ligno-cellulosic seed fibre from *Wrightia tinctoria* has been carried out (Subramanian *et al.*, 2005).

The bark is used as stomachic and in the treatment of abdominal pain and skin diseases (Shah and Gopal, 1988), as antidiarrhoeal and antihemorrhagic. Different extracts of leaf parts of *Wrightia tinctoria* and fruit powder of *Morinda citrifolia*

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have been studied against replication of HIV-1 (Selvam *et al.*, 2009). The ethanol extract of bark is used in wound healing in rats (Veerapur *et al.*, 2004). The leaf extracts showed the antibacterial activity against skin microorganisms (Kannan and Shanmugavadivu, 2006). The plant is used in Ayurveda, Unani and Siddha medicines as astringent, febrifuge and tonic. The seeds are said to possess antipyretic, analgesic and anti-inflammatory activity.

But these studies are not enough for identifying and characterizing the bioactive compounds in this plant. The purpose of this study was to identify and characterize the bioactive principles from the root bark of *Wrightia tinctoria*.

## MATERIALS AND METHODS

**Preparation of plant material:** The woody stem of *Wrightia tinctoria* were collected during the month of May-June, 2007 from the Toranmal Hills of Maharashtra. The plant was taxonomically identified by Professor Dr. D.A. Patil, HOD Botany Department, SSVPS College, Dhule, North Maharashtra University, Jalgaon. The dried woody stem powder (4 kg) was subjected to hot extraction with petroleum ether by Soxhlet extractor and after evaporation of the solvent 16 g extract was found. The extract then saponified with alcoholic KOH, to remove fatty material yields 10 g of unsaponified matter.

**Isolation and purification of compounds:** A small quantity of unsaponifiable matter was dissolved in chloroform and this solution was spotted on TLC plates. Then the TLC plates were run by specific solvent system and were viewed individually under UV light and also with the vanillin-H<sub>2</sub>SO<sub>4</sub> reagent. Through several pilot experiments, it was found that the compounds of unsaponifiable fraction were separated by the solvent system of petroleum ether and ethyl acetate in the proportion of 7: 3. The unsaponifiable fraction, 8 g was subjected to column chromatography on a silica gel (60-120 mesh) with gradient elution using petroleum ether: ethyl acetate (Stahl, 1969).

Three fractions were found homogeneous on TLC plate by using petroleum ether: ethyl acetate (7:3), petroleum ether: chloroform (10: 1), n hexane: ethyl acetate (9: 1) and petroleum ether: methanol (9:1) solvent systems. These fractions were crystallized (Bahl and Bahl, 1992) and named as PEW-1 (Pet Ether Wrightia-1), PEW-2 (Pet Ether Wrightia -2) and PEW-3 (Pet Ether Wrightia-3), respectively.

**Test for alcohol:** Four gram of ceric ammonium nitrate was dissolved in 10 mL of 2 N HNO<sub>3</sub>, on mild heating. A few

crystals of PEW-2 and PEW-3 were dissolved in 0.5 mL of dioxane. The solution was added to 0.5 mL of ceric ammonium nitrate reagent and diluted to 1 mL with dioxane and shaken well. All PEW-1, PEW-2 and PEW-3 developed yellow to red color indicating the presence of an alcoholic hydroxyl group (Harborne, 1998).

### Test for steroid

**Salkowski reaction:** A few crystals of PEW-1, PEW-2 and PEW- 3 were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to the solution. For all PEW-1, PEW -2 and PEW-3, a reddish color was seen in the upper chloroform layer (Harborne, 1998).

**Liebermann-burchard reaction:** A few crystals of PEW-1, PEW-2 and PEW-3 were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to it followed by the addition of 2-3 drops of acetic anhydride. Solution for all PEW-1, PEW-2 and PEW-3 turned violet blue and finally green (Harborne, 1998).

**Spectroscopic characterization:** Different spectroscopic methods were used to elucidate the structure of PEW-1, PEW-2 and PEW-3. Among the spectroscopic techniques UV, IR, <sup>1</sup>H-NMR, <sup>13</sup>CNMR and GCMS were carried out. The ultraviolet spectrum was recorded on 1601 UV-Visible spectrophotometer (Shimadzu), infra red spectrum was recorded on FTIR 8400 s (Shimadzu) at NMU, Jalgaon, MS, India. <sup>1</sup>HNMR spectra were recorded on a Varian-500 MHZ NMR spectrometer (Shimadzu) at Cadila Pharmaceuticals, Ahmedabad, India and <sup>13</sup>CNMR spectra were recorded on a Varian-100 MHZ NMR spectrometer (Shimadzu) at CDRI, Lucknow, India. The <sup>1</sup>HNMR and <sup>13</sup>CNMR spectra were recorded using CDCl<sub>3</sub>, as solvent with Tetramethylsilane (TMS) as an internal standard. Mass spectrum was recorded at high resolution on a mass spectrometer (Perkin Elmer Autosystem XL with Turbomass) at Sophisticated Instrumentation Centre for Applied Research and Technology, Anand, Gujarat India and the data are given in mlz values.

## RESULTS

From the positive tests for steroids and triterpenoids given by the PEW-2, PEW-3 and PEW-1, they were assumed to be a triterpenoid and sterols. The melting point of PEW-1 was 213°C; the UV  $\lambda_{max}$  value of PEW-1 was 350 nm. Mass spectrum of PEW-1 showed parent molecular ion [M<sup>+</sup>] peak at mlz 426 which corresponds to the molecular formula C<sub>30</sub>H<sub>50</sub>O (Fig. 1).

The melting point of PEW-2 was 165°C; the UV  $\lambda_{max}$  value of PEW-2 was 257 nm. Mass spectrum of PEW-2 showed parent molecular ion [M<sup>+</sup>] peak at mlz 412 which corresponds to the molecular formula C<sub>29</sub>H<sub>48</sub>O (Fig. 2).

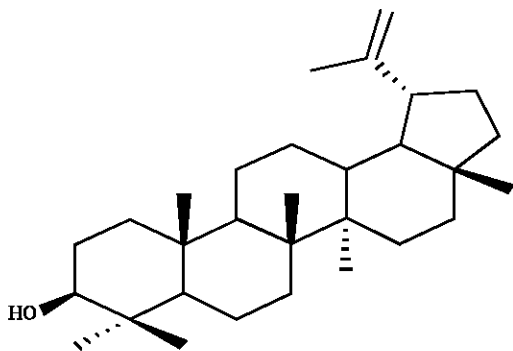


Fig. 1: The chemical structure of PEW-1 (Lupeol)

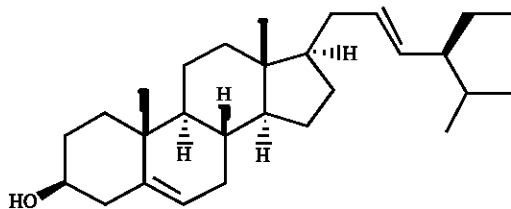


Fig. 2: The chemical structure of PEW-2 (Stigmasterol)

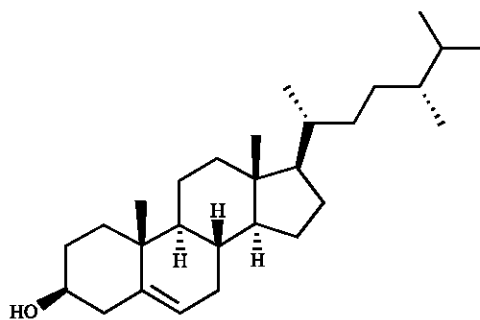


Fig. 3: The chemical structure of PEW-3 (Campesterol)

The melting point of PEW-3 was 150°C, the UV  $\lambda_{\max}$  value of and PEW-3 was 251 nm. Mass spectrum of PEW-3 showed a parent molecular ion [ $M^+$ ] peak at m/z 400 which corresponds to the molecular formula and  $C_{28}H_{48}O$  (Fig. 3).

In the IR spectrum of PEW-1, PEW-2 and PEW-3, an intensely broad band at 331, 3320 and 3320  $cm^{-1}$ , showed presence of OH stretching and in the  $^1H$ -NMR spectrum of PEW-1, H-3 proton appeared as a triplet of a double doublet (tdd) at  $\delta$  3.21, H-29 proton gives two multiplets at  $\delta$  4.71 and  $\delta$  4.56 (Table 1) and of PEW-2, H-3 proton appeared as a triplet of a double doublet (tdd) at  $\delta$  3.62 and, H-6 olefinic proton showed a multiplet at  $\delta$  5.14. Two olefinic protons appeared downfield at  $\delta$  4.16 m and  $\delta$  4.14 m. Six methyl proton appeared at

Table 1: Spectroscopic data of PEW-1 (Lupeol)

Spectroscopic technique	Data
UV $\lambda_{\max}$ :	350 nm
IR: (CHCl <sub>3</sub> ):	3311, 2946, 2870, 1638, 1464, 1189, 1035, 996, 680 $cm^{-1}$
$^1H$ NMR (CDCl <sub>3</sub> ):	$\delta$ 4.71 (s, 1H, H-29), $\delta$ 4.56 (s, 1H, H-27), $\delta$ 3.21 (d, 1H, H-3), $\delta$ 2.37 (m, 1H, H-19), $\delta$ 1.91 (m, 1H, H-21), $\delta$ 1.69 (s, 3H, H-30), $\delta$ 1.67 (t, 1H, H-13), 1.61 (s, 3H, H-2A), $\delta$ 1.54 (s, 3H, H-2B), $\delta$ 1.53 (d, 1H, H-11), $\delta$ 1.42 (d, 1H, H-14) $\delta$ 1.39 (q, 1H, H-6), $\delta$ 1.33 (s, 1H, H-21), $\delta$ 1.29 (q, 1H, H-12), $\delta$ 1.28 (s, 1H, H-9), $\delta$ 1.04 (s, 1H, H-23), $\delta$ 1.01 (d, 1H, H-15) $\delta$ 0.98 (s, 3H, H-23), $\delta$ 0.97 (s, 3H, H-27), $\delta$ 0.91 (t, 1H, H-18) $\delta$ 0.84 (s, 3H, H-25), $\delta$ 0.79 (s, 3H, H-28), $\delta$ 0.69 (d, 1H, H-5)
$^{13}C$ NMR (CDCl <sub>3</sub> ):	$\delta$ 150.80, $\delta$ 110.10, $\delta$ 78.60, $\delta$ 55.10, $\delta$ 48.60, $\delta$ 42.28, $\delta$ 41.28, $\delta$ 36.40, $\delta$ 33.40, $\delta$ 31.10, $\delta$ 27.90, $\delta$ 25.10, $\delta$ 22.80, $\delta$ 19.10, $\delta$ 14.80
GCMS: m/z with % abundance	426 (M <sup>+</sup> , C <sub>30</sub> H <sub>50</sub> O), 43 (100), 382 (6), 367 (4), 261 (14), 213 (15), 161 (16), 55 (100), 408 (9), 393 (9), 365 (14), 297 (8), 218 (68), 203 (37), 189 (50), 175 (37), 161 (41), 147 (48), 135 (64), 121 (71), 95 (78), 81 (36), 69 (68), 41 (64)

Table 2: Spectroscopic data of PEW-2 (Stigmasterol)

Spectroscopic technique	Data
UV $\lambda_{\max}$ :	257 nm
IR: (CHCl <sub>3</sub> ):	3320, 2946, 2854, 1480, 1388, 1189, 1096, 1035, 668 $cm^{-1}$
$^1H$ NMR (CDCl <sub>3</sub> ):	$\delta$ 5.14 (m, 1H, H-6), $\delta$ 4.16 (s, 1H), $\delta$ 4.14 (s, 1H), $\delta$ 3.62 (tdd, OH, H-3), $\delta$ 1.27 (s, 3H), $\delta$ 1.19 (s, 3H), $\delta$ 1.07 (s, 3H), $\delta$ 0.99 (s, 3H), $\delta$ 0.91 (s, 3H)
$^{13}C$ NMR (CDCl <sub>3</sub> ):	$\delta$ 140.80, $\delta$ 130.10, $\delta$ 128.60, $\delta$ 121.80, $\delta$ 71.60, $\delta$ 58.10, $\delta$ 56.10, $\delta$ 52.10, $\delta$ 50.10, $\delta$ 42.28, $\delta$ 40.28, $\delta$ 37.40, $\delta$ 33.40, $\delta$ 31.71, $\delta$ 28.40, $\delta$ 27.10, $\delta$ 24.10, $\delta$ 21.80, $\delta$ 19.10, $\delta$ 17.10, $\delta$ 15.10, $\delta$ 12.80
GCMS: m/z with % abundance	412 (M <sup>+</sup> , C <sub>28</sub> H <sub>48</sub> O), 55 (100), 394 (8), 255 (16), 213 (9), 199 (8), 159 (25), 145 (29), 133 (26), 121 (19), 105 (32), 91 (34), 83 (64), 81 (59), 69 (52), 41 (39)

Table 3: Spectroscopic data of PEW-3 (Campesterol)

Spectroscopic technique	Data
UV $\lambda_{\max}$ :	251 nm
IR: (CHCl <sub>3</sub> ):	3320, 2946, 1464, 1189, 1096, 1042 and 680 $cm^{-1}$
$^1H$ NMR (CDCl <sub>3</sub> ):	$\delta$ 5.10 (m, 1H, H-6), $\delta$ 3.21 (tdd, OH, H-3), $\delta$ 1.27 (s, 3H), $\delta$ 1.14 (s, 3H), $\delta$ 1.09 (s, 3H), $\delta$ 0.98 (s, 3H), $\delta$ 0.95 (s, 3H)
$^{13}C$ NMR (CDCl <sub>3</sub> ):	$\delta$ 140.80, $\delta$ 121.10, $\delta$ 71.60, $\delta$ 56.10, $\delta$ 50.10, $\delta$ 42.28, $\delta$ 37.40, $\delta$ 33.40, $\delta$ 31.71, $\delta$ 28.10, $\delta$ 25.10, $\delta$ 21.80, $\delta$ 15.10, $\delta$ 12.80
GCMS: m/z with % abundance	400 (M <sup>+</sup> , C <sub>28</sub> H <sub>48</sub> O), 43 (100), 382 (6), 367 (4), 261 (14), 213 (15), 161 (16), 159 (19), 147 (28), 145 (37), 105 (38), 91 (37), 81 (50), 67 (32), 57 (41), 55 (62) and 29 (16)

$\delta$  1.27,  $\delta$  1.19,  $\delta$  1.07,  $\delta$  1.00,  $\delta$  0.98 and  $\delta$  0.91 for methyl group (Table 2) and of PEW-3, H-3 proton appeared at  $\delta$  3.21 as a triplet of a double doublet H-6 olefinic proton showed a multiplet at  $\delta$  5.10 and Six methyl proton appeared at  $\delta$  1.27,  $\delta$  1.14,  $\delta$  1.09,  $\delta$  1.00,  $\delta$  0.98 and  $\delta$  0.95 for methyl group (Table 3). From the above observations, PEW-1, PEW-2 and PEW-3 were found to be lupeol, stigmasterol and campesterol, respectively.

## DISCUSSION

In IR spectrum of PEW-1, a very intensely broad band at  $3319\text{ cm}^{-1}$  and moderately intense band at  $1198$  and  $680\text{ cm}^{-1}$  were observed for the O-H bond vibrations of hydroxyl group. The out of plane C-H vibrations of the unsaturated part was observed at  $822\text{ cm}^{-1}$ . The corresponding C=C vibrations was shown around  $1660\text{ cm}^{-1}$  as weakly intense band. The stretching and bending vibrations of methyl part were noticed by the intense band  $2923\text{ cm}^{-1}$  and medium intensity band at  $1466\text{ cm}^{-1}$ . The vibration of the methylenic part was shown by the band at  $2850\text{ cm}^{-1}$  and the medium band at  $1440\text{ cm}^{-1}$ . The moderately intense band at  $750\text{ cm}^{-1}$  was attributed to the rocking movement of methylenic part. The corresponding C-C vibration was shown as weak intense band at  $1042\text{ cm}^{-1}$ . In  $^1\text{H-NMR}$  spectrum of PEW-1, H-3 proton appeared as a triplet of a double doublet (tdd) at  $\delta\ 3.21$  ( $J = 4.5$  and  $1.1$  MHz) and H-29 olefinic proton showed a multiplet at  $\delta\ 4.71$  and  $\delta\ 4.56$ , respectively. Seven methyl protons also appeared at  $\delta\ 1.28$ ,  $\delta\ 1.04$ ,  $\delta\ 1.07$ ,  $\delta\ 1.01$ ,  $\delta\ 0.98$ ,  $\delta\ 0.97$  and  $\delta\ 0.91$  (3H each, s,  $\text{CH}_3$ ). These assignments are in good agreement for the structure of lupeol (Imam *et al.*, 2007).

In IR spectrum of PEW-2, a very intensely broad band at  $3320\text{ cm}^{-1}$  and moderately intense band at  $11189$  and  $680\text{ cm}^{-1}$  were observed for the O-H bond vibrations of hydroxyl group. The out of plane C-H vibrations of the unsaturated part was observed at  $890\text{ cm}^{-1}$ . The corresponding C=C vibrations was shown around  $1648\text{ cm}^{-1}$  as weakly intense band. The stretching and bending vibrations of methyl part were noticed by the intense band  $2946\text{ cm}^{-1}$  and medium intensity band at  $1480\text{ cm}^{-1}$ . The vibration of the methylenic part was shown by the band at  $2854\text{ cm}^{-1}$  and the medium band at  $1450\text{ cm}^{-1}$ . The moderately intense band at  $730\text{ cm}^{-1}$  was attributed to the rocking movement of methylenic part. The corresponding C-C vibration was shown as weak intense band at  $1035\text{ cm}^{-1}$ . In  $^1\text{H-NMR}$  spectrum of PEW-2, H-3 proton appeared as a triplet of a double doublet (tdd) at  $\delta\ 3.62$  ( $J = 4.5$  and  $1.1$  MHz) and H-6 olefinic proton showed a multiplet at  $\delta\ 5.14$ . Two olefinic protons appeared downfield at  $\delta\ 4.16$  (m) and  $\delta\ 4.14$  (m) which were identical with the chemical shift of H-22 and H-23, respectively of stigmaterol (Li *et al.*, 2006). Six methyl protons also appeared at  $\delta\ 1.27$ ,  $\delta\ 1.19$ ,  $\delta\ 1.07$ ,  $\delta\ 1.00$ ,  $\delta\ 0.99$  and  $\delta\ 0.91$  (3H each, s,  $\text{CH}_3$ ). These assignments are in good agreement for the structure of stigmaterol (Habib *et al.*, 2007; Jain and Bari, 2009).

Similarly from the IR spectrum of PEW-3, a very intensely broad band at  $3320\text{ cm}^{-1}$  and moderately intense

band at  $1189$  and  $670\text{ cm}^{-1}$  were observed for the O-H bond vibrations of hydroxyl group. The out of plane C-H vibrations of the unsaturated part was observed at  $870\text{ cm}^{-1}$ . The corresponding C=C vibrations was shown around  $1640\text{ cm}^{-1}$  as weakly intense band. The stretching and bending vibrations of methyl part were noticed by the intense band  $2946\text{ cm}^{-1}$  and medium intensity band at  $1464\text{ cm}^{-1}$ . The vibration of the methylenic part was shown by the band at  $2854\text{ cm}^{-1}$  and the medium band at  $1470\text{ cm}^{-1}$ . The moderately intense band at  $720\text{ cm}^{-1}$  was attributed to the rocking movement of methylenic part. The corresponding C-C vibration was shown as weak intense band at  $1042\text{ cm}^{-1}$ . The  $^1\text{H-NMR}$  data of PEW-3 it was seen that H-3 proton appeared at  $\delta\ 3.21$  as a triplet of a double doublet with a J value of  $4.5$  and  $1.1$  MHz and H-6 olefinic proton showed a multiplet at  $\delta\ 5.10$ . Moreover, Six methyl proton appeared at  $\delta\ 1.27$ ,  $\delta\ 1.14$ ,  $\delta\ 1.07$ ,  $\delta\ 1.00$ ,  $\delta\ 0.98$  and  $\delta\ 0.95$  (3H each, s,  $\text{CH}_3$ ). These assignments are in good agreement for the structure of campesterol (Habib *et al.*, 2007). The  $^{13}\text{CNMR}$  data of the PEW-1, PEW-2 and PEW-3 were also quite similar with the data in the literature of lupeol, stigmaterol and campesterol, respectively (Conolly and Hill, 1994).

## CONCLUSION

From these physical, chemical and spectral evidences PEW-1, PEW-2 and PEW-3 were confirmed as Lupeol (Fig. 1), Stigmaterol (Fig. 2) and campesterol (Fig. 3).

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