Pakistan Journal of Pharmaceutical Sciences
Vol.15, No.2, July 2002, pp.1-12

CHEMICAL CONSTITUENTS OF *TAGETES PATULA* L.

HUSAN BANO, SYED WASEEMUDDIN AHMED, IQBAL AZHAR, M. SHAIQ ALI*, AND NASEER ALAM*
Department of Pharmacognosy, Faculty of Pharmacy
**HEJ Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan*

ABSTRACT

Tagetes patula (Asteraceae) is a medicinal plant which is indigenous to Tropical America but cultivated in Pakistan. During the chemical study, conducted on the different parts of *T. patula* (roots, leaves and flowers) are found thiophenes, steroidal and terpenoidal type of constituents. Their structures were characterized by different spectroscopic. Among the thiophenes triterpens and steroids are two thiophenes, one triterpene and one steroid first time isolated from the genes Tegetes methods.

INTRODUCTION

Tagetes is a genus of herbs commonly known as Marigolds is a native of Mexico and other warmer parts of America and naturalized elsewhere in the tropics and subtropics. Several species are grown in gardens for ornament. The name Marigold is however, indiscriminately applied to several other genera of Compositae with golden or yellow capitula. Five species have been introduced into the Indian gardens and almost all of them are met with as escapes (Heywood V.H. and Harborne J.B. ed., 1977). The flower-heads are much used for garlands. Many *Tagetes* spp. yield strongly aromatic essential oils, all of which are known as Tagetes Oil. The oil is obtained from the entire aerial part of the plant by steam distillation for 3-4 hours and absorbing the distillate in petroleum ether or benzene; prolonged distillation spoils the aroma. The leaves, especially the tender ones, are the richest in oil.

Tagetes oil is commercially produced from *T. minuta* and *Tagetes patula* in France, and also from the former in Kenya and Australia. The tagetes oil is used for the compounding of high-grade perfumes. It is also a fly-repellent and is credited with larvicidal properties (Heywood V.H. and Harborne J.B. ed., 1977).

***Tagetes patula* Linn. (Asteraceae)**

It is commonly known as *Genda* and its English name is French Marigold. Flowering occurs in December and January. It is a common garden plant.

This is a bushy annual, 30-45 cm high, native to Mexico, and is cultivated in garden all over India as ornamental plant, upto altitude of 1,350 m having leaves 5-10 cm long, pinnately dissected segments 1-3 cm long, linear-lanceolate, serrate; flower head solitary with reddish yellow or orange-colour.

The entire aerial parts of the plant and occasionally the flowers are distilled to yield a yellowish-amber coloured essential oil which is mobile when freshly distilled (The Wealth of India, 1976).

MATERIAL AND METHODS

Plant Material

Tagetes patula roots, leaves and flowers were collected in 1996 from the Faculty of Pharmacy, University of Karachi and the plant was identified by Dr. Saood Umer, Department of Botany, University of Karachi.

Extraction and Isolation of Thiophenes

The air-dried roots of *Tagetes patula* (500 g) were chopped and soaked in ethanol for 15 days. This procedure was repeated thrice. The combined extract evaporated under vacuum at 40°C and then the gummy residue was suspended in water, then partitioned first with hexane and this hexane extract was subjected to column chromatography. Two compounds were eluted with *n*-hexane:chloroform (9:1), 5′-hydroxymethyl-5-(3-butene-1-ynyl)-2,2′-bithiophene (Heywood V.H. and Harborne J.B. ed., 1977) and another compound eluted in *n*-hexane:chloroform (8:2), 5′-methyl-5-[4-(3-methyl-1-oxobutoxy)-1-butynyl]-2,2′-bithiophene (The Wealth of India, 1976).

Extraction and Isolation of Steroids and Terpenes

The air-dried flowers (1300 g) were soaked in ethanol. Kept at room temperature for 15 days, and this procedure was repeated thrice. The combined ethanolic extract was evaporated under reduced pressure. The ethanolic gummy residue (64.4 g), so obtained, was suspended in water, then partitioned first with hexane, and thereafter with ethylacetate in order to remove neutral substances and other impurities. The aqueous portion was extracted several times with presaturated *n*-BuOH. The *n*-BuOH fractions were pooled together and evaporated under high vacuum. Each extract, *i.e. n*-hexane, ethyl acetate extract and *n*-BuOH extract were examined by thin layer chromatography (TLC). Preliminary chemical screening (chromogenic reactions on TLC plates) of hexane extracts with ceric ammonium sulphate showed sterols constituents and thiophenes and flavonoids.

The *n*-hexane fraction of flower was subjected to column chromatography on silica gel using a mixture of solvent systems; which afforded four compounds i.e. β-sitosterol in *n*-hexane-ethyl acetate (9:1); stigmasterol in *n*-hexane:ethylacetate (7:3) whereas some semipure fractions obtained by the same column, which were mixed together and further subjected to column chromatography and isolated cholesterol and lupeol in *n*-hexane:ethylacetate (6:4), *n*-hexane:ethylacetate (5:5) respectively.

Characterization of Compound 1

Hexane soluble part of the ethanolic extract of roots of *Tagetes patula* was chromatographed on silica gel using different proportions of hexane and ethylacetate as eluent. Elution with 10% chloroform in hexane yielded a semi-pure fraction which is purified by repeated column chromatography on silica gel with chloroform:hexane (4:1, 26 mg) gave compound 1 as yellow needle-like crystals.

UV λ_{max} (Et_2O): 347.2 nm.
EIMS *m/z* (rel. int. %): 245.7 [M^+] (94.6), 228.8 (70), 216.8 (100).
HREIMS *m/z*: 246.0155 [$C_{13}H_{10}OS_2$].
^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.77 (2H, d, *J*=0.8 Hz, H-6′), 5.52 (1H, dd, *J*=2.0, 11.1 Hz, H-9 (*cis*)), 5.69 (1H, dd, *J*=2.0, 17.5 Hz, H-9 (*trans*)), 6.00 (1H, dd, *J*=11.1, 17.5 Hz, H-8), 6.88 (1H, dq, *J*=0.8, 3.6 Hz, H-4′), 6.99 (1H, d, *J*=3.8 Hz, H-3), 7.00 (1H, d, *J*=3.6 Hz, H-3′), 7.08 (1H, d, *J*=3.8 Hz, H-4).
The ^{13}C-NMR ($CDCl_3$, 100 MHz) spectral data is presented in Table 1.

Characterization of Compound 2

Compound 2 was isolated from the hexane soluble part of the ethanolic extract of *Tagetes patula* roots. It was eluted from the silica gel column with 20% chloroform in hexane and was finally purified by repeated column chromatography on silica gel (hexane:chloroform, 1:1) afforded as yellowish oil (27 mg).

UV λ_{max} (Et_2O): 339 nm (ε 26, 726).
IR ν_{max} (CCl_4): 2850 (C–H stretching), 2300 (C≡C), 1717 (ester carbonyl), 1600 (C=C) cm^{-1}.
EIMS *m/z* (rel. int. %): 332.0903 [$C_{18}H_{20}O_2S_2$, M^+] (19), 230.0186 [M-$C_5H_{10}O_2$] (100), 217 (9), 197 (5), 115 (7), 102 (4).
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.97 (6H, d, *J*=6.5 Hz, H-4a), 2.12 (1H, m, H-3a), 2.22 (2H, d, *J*=6.5 Hz, H-2a), 2.48 (3H, d, *J*=1.0 Hz, H-6′), 2.78 (2H, t, *J*=6.7 Hz, H-3′′), 4.24 (2H, t, *J*=6.7 Hz, H-4′′), 6.64 (1H, dq, *J*=1.0, 3.5 Hz, H-4′), 6.88 (1H, d, *J*=3.7 Hz, H-3), 6.93 (1H, d, *J*=3.5 Hz, H-3′), 6.99 (1H, d, *J*=3.7 Hz, H-4).
The ^{13}C-NMR ($CDCl_3$, 100 MHz) spectral data is presented in Table 2.

Characterization of Compound 3

The compound 3 was also isolated from the hexane soluble part of flower of *T. patula*. The semi-pure sample 3 was further purified by washing the solid material with methanol. After washing, with methanol compound 3 was obtained as needles (20 mg).

EIMS: *m/z* 386 (M^+, $C_{27}H_{46}O$), 368 $(M\text{-}H_2O)^+$, 353 [M^+, (H_2O+CH_3)], 273 (M^+-side chain), 255 [M^+-(H_2O+side chain)], 213 (M^+-$C_{11}H_{55}O$), 145 and 119.
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 3.51 (*m,* W½ = 15.20 Hz, H-3), 5.35 (distorted *t*, H-6), 0.67 (*s*, H-18), 1.00 (*s*, H-19), 0.91 (*d, J*=6.56 Hz, H-21), 0.86 (*d, J*=6.64 Hz, H-26 and H-27).
The ^{13}C-NMR ($CDCl_3$, 75.4 MHz) spectral data is presented in Table 3.

Characterization of Compound 4

Compound 4 was obtained from the column chromatography (silica gel, 230-400 mesh size, E. Merck) of the hexane soluble part of the ethanolic extract of *T. patula* flowers. It got crystallized upon evaporation of the fractions obtained from elution of the silica gel column with 10% ethyl acetate in hexane. It was further purified upon recrystallization from chloroform.

Melting point: 135°C.
Optical rotation $[\alpha]_D^{25}$: -35.5° ($CHCl_3$, c=0.1429).
EIMS *m/z* (rel. int. %): 414 [M^+] (15), 399 (10), 396 (12), 381 (72), 329 (25), 303 (23), 275 (12), 255 (30).
HREIMS *m/z*: 414.4091 [$C_{25}H_{50}O$].
IR ν_{max} ($CHCl_3$): 3450, 3050, 1650, 815 cm^{-1}.
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 5.23 (1H, m, H-6), 3.32 (1H, m, H-3), 1.01 (3H, s, H-19), 0.92 (3H, d, *J*=6.2 Hz, H-21), 0.84 (3H, t, *J*=7.0 Hz, H-29), 0.83 (3H, d, *J*=6.5 Hz, H-26), 0.81 (3H, d, *J*=6.5 Hz, H-27), 0.68 (3H, s, H-18).
The ^{13}C-NMR ($CDCl_3$, 75.4 MHz) spectral data is presented in Table 4.

Characterization of Compound 5

The fraction eluted with hexane-ethyl acetate (7:3), was again applied to silica gel column chromatography which gave a white powdery material identified through spectroscopy as stigmasterol.

Melting point: 170°C.

Optical rotation $[\alpha]_D^{20}$: –51° ($CHCl_3$, c=0.21).

EI-MS *m/z*: 412 [$C_{29}H_{48}O$, M^+], 397 [M-Me]$^+$, 394 [M-H_2O]$^+$, 379 [M-Me-H_2O]$^+$, 369 [M-C_3H_7]$^+$, 351 [M-C_3H_7-H_2O]$^+$, 300 [M-C_8H_{16}]$^+$, 273 [M-H_2O-C_9H_{13}]$^+$ and 271 [M-$C_{10}H_{19}$+2H (entire substituent at C-17)]$^+$.

IR ($CHCl_3$) ν_{max}: 3400 (OH), 3025, 1410, 1250, 690 and 820 cm^{-1} (C=C).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 5.33 (1H, m, H-6), 5.15 (2H, m, H-22 and H-23), 3.21 (1H, m, H-3), 0.90 (3H, d, *J*=6.5 Hz, Me-21), 0.83 (3H, d, *J*=6.6 Hz, Me-26), 0.84 (3H, t, *J*=7.0 Hz, Me-29), 0.81 (3H, d, *J*=6.5 Hz, Me-27), 0.80 (3H, s, Me-19) and 0.65 (3H, s, Me-18).

The ^{13}C-NMR ($CDCl_3$, 100 MHz) spectral data is presented in Table 5.

Characterization of Compound 6

The fraction eluted with hexane-ethyl acetate (5:5) from hexane soluble part of the ethanolic extract of *T. patula* flowers was subjected to repeated column chromatography, consequently furnishing a compound identified as lupeol.

Melting point: 213°C.

Optical rotation $[\alpha]_D^{20}$: +26.5° ($CHCl_3$, c=0.03).

EI-MS *m/z*: 426 [$C_{30}H_{50}O$, M^+], 411 [M-Me]$^+$, 408 [M-H_2O]$^+$, 393 [M-Me-H_2O]$^+$, 385 [M-41]$^+$, 220 [M-$C_{15}H_{26}$]$^+$, 218 [M-$C_{14}H_{24}O$]$^+$, 207 [M-$C_{16}H_{27}$]$^+$, 189 [M-$C_{16}H_{29}O$]$^+$ and 139 [M-$C_{21}H_{35}$]$^+$.

IR ($CHCl_3$) ν_{max}: 3450 (–OH), 3075, 1645 and 880 cm^{-1} (terminal C=C).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 4.61-4.75 (1H each br.s, H_2-29), 3.21 (1H, dd, *J*=9.8, 4.2 Hz, H-3), 1.05 (3H, s, Me-26), 0.97 (3H, s, Me-23), 0.94 (3H, s, Me-27), 0.85 (3H, s, Me-25), 0.79 (3H, s, Me-28) and 0.77 (3H, s, Me-24).

The ^{13}C-NMR ($CDCl_3$, 100 MHz) spectral data is presented in Table 6.

Instrumentation for Phytochemical Analysis

All melting points were recorded in glass capillary tubes using Büchi melting point apparatus. Optical rotations were measured on JASCO DIP-360 (Japan Spectroscopic Co. Ltd., Tokyo, Japan) digital polarimeter.

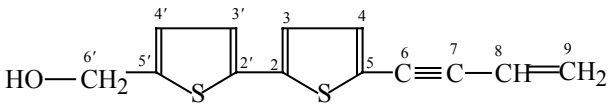
The UV spectra were recorded on Shimadzu UV-240 (Shimadzu Corporation, Kyoto, Japan) spectrophotometer. The IR spectra were recorded on JASCO IRA-1 (Japan Spectroscopic Co. Ltd., Tokyo, Japan) and Shimadzu IR-460 (Shimadzu Corporation, Kyoto, Japan) instrument. The Nuclear Magnetic Resonance (NMR) were recorded on Bruker AM 300 FT NMR, AM 400 FT NMR and AM 500 FT NMR spectrometers using TMS as internal standard. The ^{13}C-NMR spectra were recorded at 75, 100 and 125 MHz on Bruker AM 300 FT NMR, AM 400 FT NMR and AM 500 FT NMR spectrometers respectively. Mass spectra were recorded on Varian MAT-112s and Finnigan MAT-112 and 312A double focusing mass spectrometers connected to DEC PDP 11/34 and IBM-AT compatible PC based system, respectively. Electron impact, peak matching, field desorption (FD) and fast atom bombardment (negative FAB) experiments were performed on MAT-312A or Jeol-JMS HX-110 spectrometers. FABMS were recorded in a glycerol-water (1:1) matrix in the presence of K1. High resolution electron impact mass spectra were recorded on a Jeol-JMSH×110 mass spectrometer.

Column chromatography was performed with silica gel (230-400 mesh size – E. Merck). TLC was performed on silica (GF_{254}). UV lamp (250 nm) and ceric sulphate spray were used for visualization.

RESULTS AND DISCUSSION

4.1. 5′-Hydroxymethyl-5-(3-butene-1-ynyl)-2,2′-bithiophene (1)

Hexane soluble part of the ethanolic extract of roots of *Tagetes patula* was chromatographed on silica gel using different proportions of *n*-hexane and chloroform as eluent. Elution with 10% chloroformin *n*-hexane yielded compound 1.



1

The electron impact mass spectrum (EIMS) exhibited the molecular ion peak [M^+] at *m/z* 245.7 whereas the high resolution electron impact mass spectrum (HREIMS) gave an exact value of 246.0155 which was in accordance to the molecular formula $C_{13}H_{10}OS_2$. The formula showed nine degrees of unsaturation in the compound.

The UV spectrum (Et_2O) displayed absorption maximum at 247.2 nm, characteristic of a bithiophene acetylenic chromophore conjugated with a double bond (Bohlmann F. *et al.,* 1973). The ^{1}H-NMR spectrum was very conclusive in determining the structure of compound 1. A doublet of two protons appeared at δ 4.77 (*J*=0.8 Hz) which was assigned to H-6′. Three doublet of doublets at δ 5.52 (*J*=2.0, 11.1 Hz), 5.69 (*J*=2.0, 17.5 Hz) and 6.00 (*J*=11.1, 17.5 Hz) were characteristic of an unsubstituted terminal methylene group and were assigned to H-9 (*cis*), H-9 (*trans*) and H-8 protons respectively. The presence of four protons at δ 6.88 (*dq*, *J*=0.8, 3.6 Hz), 6.99 (*d, J*=3.8 Hz), 7.00 (*d, J*=3.6 Hz) and 7.08 (*d, J*=3.8 Hz) suggested the presence of a bithiophene system in the molecule. These protons were assigned to H-4′, H-3, H-3′ and H-4, respectively. The value of the coupling constant of H-4′ proton suggested its allylic attachment to H-6′ proton.

Table 1

^{13}C-NMR ($CDCl_3$, 75 MHz) chemical shifts of compound 1

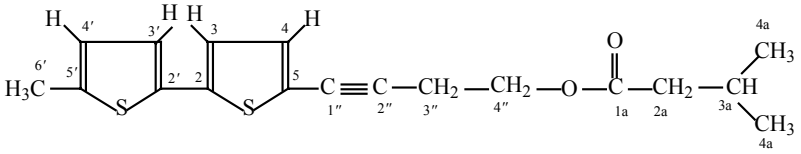
Position	Chemical shift (δ)	Multiplicity (DEPT)
2	138.5	C
3	123.4	CH
4	132.8	CH
5	121.9	C
6	83.1	C
7	93.0	C
8	116.8	CH
9	126.9	CH_2
2′	138.9	C
3′	123.8	CH
4′	126.1	CH

The ^{13}C-NMR (broad band) exhibited signals for all the thirteen carbons (Table 1). The multiplicities of the various carbons were confirmed with the help of the DEPT experiments which showed that two methylenes, five methines and six quaternary carbons were present in the molecule. The chemical shifts of the various carbon atoms (Table 1) were assigned with

comparison to the values reported in the literature (Bohlmann F. *et al.,* 1973). The compound is being first time reported from the genus *Tagetes*.

Methyl-5-[4-(3-methyl-1-oxobutoxy)-1-butynyl]-2,2′-bithiophene(2)

The compound 2 was obtained from the *n*-hexane soluble part of the ethanolic extract of roots of *Tagetes patula*. When the *n*-hexane extract was subjected to column chromatography it afforded a fraction upon elution with *n*-hexane:chloroform (80:20). Further purification of this fraction by repeated column chromatography afforded a pure compound in oily form (chloroform:*n*-hexane, 1:1) (27 mg).



2

This oil was labile in nature. The EIMS showed the molecular ion peak [M^+] at *m/z* 332.06. Presence of sulphur in the compound was detected by the appearance of a strong [M^{+2}] peak at *m/z* 334.06 in the EIMS. Its intensity was found to be 11.1% of that of the molecular ion peak. Exact molecular weight was determined by peak matching where it came out to be *m/z* 332.0902, corresponding to the molecular formula $C_{18}H_{20}O_2S_2$ having nine degrees of unsaturation in the skeleton.

Table 2
^{13}C-NMR ($CDCl_3$, 75 MHz) chemical shifts of compound 2

Position	Chemical shift (δ)	Multiplicity (DEPT)
2	138.6	C
3	122.5	CH
4	132.5	CH
5	121.6	C
2′	134.5	C
3′	124.3	CH
4′	126.0	CH
5′	139.8	C
6′	15.3	CH_3
1″	75.3	C
2″	90.5	C

The IR (CCl_4) spectrum displayed intense absorption peaks at 2850 (C-H), 2300 (C≡C) and 1717 (ester carbonyl) cm^{-1}. The UV (Et_2O) spectrum showed an absorption maxima at 338.8 nm (ε° 26726). Both the ε° and the absorption maxima values suggested the presence of a 2,2′-bithiophene acetylene chromophore. The ^{1}H-NMR ($CDCl_3$, 300 MHz) displayed a 6H-doublet at δ

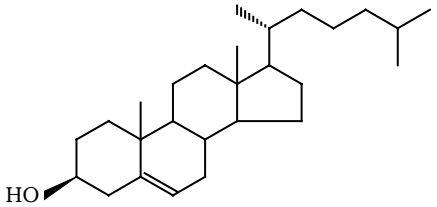
0.97 (J=6.5 Hz) for the two secondary methyls. A 1H-multiplet resonating at δ 2.12 was assigned to the C-3a methine proton, whereas a 2H-doublet at δ 2.22 (J=6.5 Hz) was due to the C-2a methylene protons. Two 2H-triplets at δ 2.78 (J=6.7 Hz) and 4.24 (J=6.7 Hz) were due to the C-3‴ and C-4‴ methylene protons, vicinally coupled to each other. Three downfield signals resonating as doublets and integrating for one proton each at δ 6.88 (J=3.5 Hz), 6.90 (J=3.5 Hz) and 6.99 (J=3.5 Hz) were ascribed to C-3, C-3′ and C-4′ methine protons, respectively. The C-4′ methine proton appeared as a doublet of quartet at δ 6.64 (J_1=1.0 Hz, J_2=3.5 Hz). The C-6′ methyl protons appeared as a 3H-doubled at δ 2.48 (J=1.0).

The broad band decoupled ^{13}C-NMR ($CDCl_3$, 100 MHz) showed resonances for all the eighteen carbons of the molecule. The DEPT (45°, 90°, 135°) spectra revealed the presence of three methyl, five methylene and three methine carbons, whereas there were found to be seven quaternary centres by subtracting DEPT from the BB.

The C-1a ester carbonyl resonated at δ 172.8, whereas the SP-hybridized C-1″ and C-2″ appeared at δ 75.3 and 90.5 respectively. The complete ^{13}C-NMR data along with multiplicities is presented in table 2. Comparison of the above stated spectral data with the reported literature confirmed its identity as being 5-methyl-5-[4-(3-methyl-1-oxobutoxy)-1-butynyl]-2,2′-bithiophene (2) (Ahmad V.U. and Alam N., 1996). This compound has been first time isolated from the genus *Tagetes*.

Cholesterol (3)

Compound 3 was obtained from the column chromatography (silica gel, 230-400 mesh size) of the *n*-hexane soluble part of the ethanolic extract of flowers of *T. patula* with 40% ethyl acetate in *n*-hexane.



3

The EI-mass spectrum of compound 3 showed the molecular ion peak at *m/z* 386. After peak matching the molecular formula was deduced as $C_{27}H_{46}O$. Another significant fragment appeared at *m/z* 368 due to the loss of water molecule from the molecular ion peak confirming the presence of at least one hydroxyl group in the molecule.

The identification of 3 was supported by proton NMR data which showed the two tertiary methyls at δ0.67(*s*) and 1.00(*s*) assigned to H-18 and H-19, respectively. In the same spectrum three secondary methyls appeared at δ0.91 (*d, J*=6.56 Hz) and 0.86 (6H, *d, J*=6.64 Hz) for H-21 and H-27, H-26, respectively. A multiplet at δ3.51 (1H, W½ = 15.20 Hz) and a distorted triplet at δ5.35 appeared in proton spectrum were characteristic signals of H-3 and H-6 respectively.

The ^{13}C-NMR spectrum (broad band) of 3 showed 27 carbon signals. The DEPT experiment clearly resolved 27 carbons into five methyls, eleven methylenes and eight methine signals. The remaining three carbon signals must be quaternary in nature. All the carbon and proton signals in

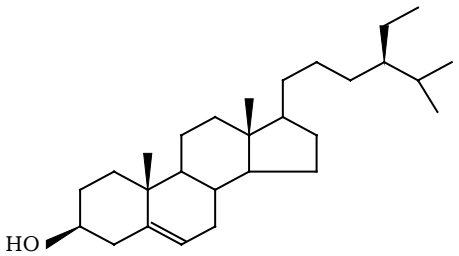
the ^{1}H- and ^{13}C spectra are in complete agreement with the structure of 3 as cholesterol. This is a new addition in the list of constituents isolated from *T. patula* (Ahmad V.U. and Alam N., 1995).

Table 3
^{13}C-NMR ($CDCl_3$, 75.4 MHz) chemical shifts of compound 3

Carbon No.	Chemical shift (δ)	Carbon No.	Chemical shift (δ)
C-1	37.14	C-15	24.19
C-2	31.53	C-16	28.12
C-3	71.74	C-17	50.06
C-4	42.16	C-18	11.74
C-5	140.68	C-19	19.27
C-6	121.67	C-20	36.08
C-7	31.79	C-21	18.60
C-8	31.80	C-22	29.34
C-9	50.03	C-23	35.68
C-10	36.39	C-24	39.41
C-11	20.98	C-25	27.91
C-12	39.68	C-26	22.44
C-13	42.18	C-27	22.70
C-14	56.68		

β-Sitosterol (24-R-stigmast-5-ene-3β-ol) (4)

β-Sitosterol was obtained from the column chromatography (silica gel) of the *n*-hexane soluble part of the ethanolic extract.



4

The melting point of 4 was found to be 135-136°C, optical rotation $[\alpha]^{25}$ –35.5 ($CHCl_3$), UV (MeOH) absorption at 250 nm and maximum absorption in the IR spectrum ($CHCl_3$) were at 3450 (OH), 3050 (C-H), 2900 (C-H) and 1680, 1460 (C=C) cm^{-1}. The EIMS displayed the molecular ion peak [M^+] at *m/z* 414. The high resolution electron impact mass spectrum (HREIMS) showed an exact value of 414.4091 which corresponded to the molecular formula $C_{29}H_{50}O$. The other prominent fragments appeared at *m/z* 399 ([M^+-CH_3]$^+$), 396 ([M^+-H_2O]$^+$), 381 ([M^+-CH_3-H_2O]$^+$), 329 ([M^+-C_5H_7-H_2O]$^+$), 303 ([M^+-C_7H_9-H_2O]$^+$), 273 ([M^+-side chain]$^+$) and 255 ([M^+-side chain-H_2O]$^+$) which were characteristic of sterol with double bond at C-5 (Rubinstein I. *et al.*, 1976).

Table 4
^{13}C-NMR ($CDCl_3$, 75.4 MHz) chemical shifts of β-sitosterol (4)

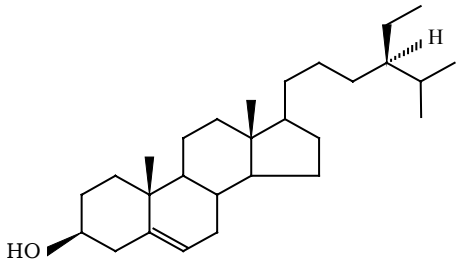
Carbon No.	Chemical shift (δ)	Carbon No.	Chemical shift (δ)
C-1	37.3	C-16	28.2
C-2	31.8	C-17	56.2
C-3	71.9	C-18	11.9
C-4	42.4	C-19	19.4
C-5	140.9	C-20	36.2
C-6	121.8	C-21	19.4
C-7	32.0	C-22	34.0
C-8	32.0	C-23	29.3
C-9	50.8	C-24	50.3
C-10	36.6	C-25	26.2
C-11	21.1	C-26	18.8
C-12	40.0	C-27	19.8
C-13	42.6	C-28	23.1
C-14	56.7	C-29	11.9
C-15	24.3		

The ^{1}H-NMR of the compound exhibited five methyl signals at δ 0.76 (H-18) and 0.83-0.99 (H-21, 26, 27 and 29). The signals for H-3β and H-6 were found at δ 3.20 and 5.34 respectively. The ^{13}C-NMR spectrum showed signals for all the twenty nine carbons. The mass fragmentation pattern and ^{1}H- and ^{13}C-NMR data showed close identity of 4 to that reported for β-sitosterol (Ahmad V.U. and Alam N., 1995). It was further confirmed by co-TLC and mix melting point with an authentic sample of β-sitosterol.

Stigmasterol [24-(S)-stigmast-5,22E-dien-3β-ol] (5)

Stigmasterol was obtained as a white powdery mass from the fraction eluted with *n*-hexane:ethyl acetate (7:3) when it was subjected to column chromatography.

Structure of the compound was established on the basis of spectroscopic studies and comparison of its spectral data with the reported literature.



5

The compound when subjected to the EIMS afforded the molecular ion at *m/z* 426, corresponding to a molecular formula $C_{30}H_{50}O$ having six degrees of unsaturation in it. A peak at *m/z* 411 appeared due to the loss of a methyl group from the molecular ion, whereas the ion at *m/z* 408 was the result of the loss of a water molecule from M^+ indicating the presence of a hydroxyl in the molecule.

Table 5
^{13}C-NMR ($CDCl_3$, 75.4 MHz) chemical shifts of stigmasterol (5)

Carbon No.	Chemical shift (δ)	Carbon No.	Chemical shift (δ)
C-1	37.42	C-16	28.90
C-2	31.81	C-17	56.00
C-3	71.92	C-18	12.43
C-4	42.40	C-19	19.40
C-5	140.92	C-20	40.50
C-6	121.78	C-21	21.12
C-7	31.91	C-22	138.40
C-8	31.89	C-23	129.48
C-9	50.37	C-24	51.31
C-10	36.61	C-25	32.00
C-11	21.01	C-26	19.00
C-12	39.78	C-27	21.21
C-13	42.40	C-28	25.41
C-14	57.00	C-29	12.00
C-15	24.41		

The IR ($CHCl_3$) spectrum afforded absorption bands at 3400, 3025, 1410, 1250, 690 and 820 cm^{-1} inferring the presence of hydroxyl and olefinic functionalities in it. The UV spectrum exhibited only terminal absorption indicating lack of any conjugated chromophore.

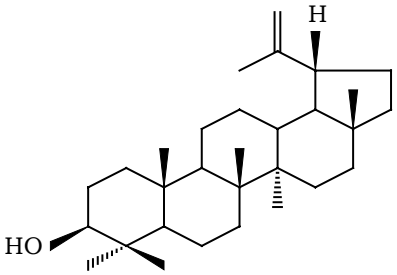
The ^{1}H-NMR ($CDCl_3$, 300 MHz) showed signals for all the protons. It displayed two 3H-singlets at δ 0.65 and 0.80 for the two tertiary methyls CH_3-18 and CH_3-19 respectively.

Three 3H-doublets resonating at δ 0.81 (*J*=6.5 Hz), 0.83 (*J*=6.6 Hz) and 0.90 (*J*=6.5 Hz) were ascribed to the three secondary methyls i.e. CH_3-27, CH_3-26 and CH_3-21 respectively. The only primary methyl, CH_3-29 appeared as a triplet at δ 0.84 (*J*=7.0 Hz). A 1H-multiplet resonating at δ 3.21 was due to the H-3 germinal to a hydroxy group. A 2H-multiplet at δ 5.15 was due to the H-22 and H-23 olefinic protons whereas the H-6 olefinic proton.

The ^{13}C-NMR ($CDCl_3$, 100 MHz) displayed signals for all the twenty nine carbons. Carbon numbers and their corresponding ^{13}C-chemical shifts are presented in table 5. Further confirmation of the compound was made by comparing the mixed melting points with authentic sample and also on TLC. The spectroscopic data was found to be identical with stigmasterol as reported in literature (Ahmad V.U. and Alam N., 1995).

Lupeol (6) [Lup-20-(2a)-en-3β-ol)

After compound 6, another ceric sulphate active compound (6) was eluted from the same column with 50% in hexane.



6

It showed a molecular ion peak in its electron impact mass spectrum at *m/z* 426 which showed an exact value of 426.6998 in high resolution mass spectrum, corresponding to the molecular formula $C_{30}H_{50}O$. The IR spectrum displayed strong absorption bands for hydroxyl group at 3440 cm^{-1} and for $C=CH_2$ group at 3070, 1650 and 880 cm^{-1}. The ^{1}H-NMR spectrum revealed the presence of seven tertiary methyl groups (singlets at δ 0.76, 0.85, 0.90, 0.96X2, 1.05 and 1.59), along with a multiplet at δ 4.63 which was assigned to the olefinic protons. The signal at δ 3.65 (*dd, J*=4.2, 10.6 Hz) was attributed to a proton geminal to alcoholic group. Further information about the structure of the compound was provided by its mass spectrum which exhibited typical fragmentation pattern for pentacyclic triterpene of the lupane series (Silverstein R.M. *et al.,* 1977 and Budzikiewicz H. *et al.,* 1963).

Table 6

^{13}C-NMR ($CDCl_3$, 75.4 MHz) chemical shifts of lupeol (6)

Carbon No.	Chemical shift (δ)	Carbon No.	Chemical shift (δ)
C-1	38.61	C-16	35.50
C-2	27.50	C-17	42.90
C-3	78.79	C-18	48.20
C-4	38.75	C-19	47.9
C-5	55.30	C-20	150.61
C-6	18.31	C-21	29.87
C-7	34.21	C-22	39.87
C-8	40.82	C-23	28.00
C-9	50.42	C-24	15.41
C-10	37.10	C-25	16.12
C-11	20.92	C-26	15.99
C-12	25.21	C-27	14.50
C-13	38.00	C-28	18.10
C-14	42.79	C-29	109.21
C-15	27.40	C-30	19.21

The ^{13}C-NMR spectrum of the compound showed at the thirty carbon atoms. The spectral data of compound 6 was in complete agreement to that previously reported for lupeol (Budzikiewicz H. *et al.,* 1964 and Wenkert E., 1978).

This is a new entry in the list of constituents isolated from the *Tagetes patula*.

REFERENCES

Ahmad, V.U. and Alam, N. (1996). Acetylenic thiophene derivatives from Blumea oblique. *Phytochemistry,* **42**: 733-735.

Ahmad, V.U. and Alam, N. (1995). New antifungl bithienyl acytylenes from Blumea oblique. *J. Natural Products,* **58**(9): 1426-1429.

Bernard, A.A. and Tokes, L. (1977). Electron impact induced fragmentation of cholesterol and related C-5 unsaturated steroids. *J. Org. Chem.* **42**: 725-731.

Bohlmann, F., Burkhardt, T. and Zedro, C. (1973). Naturally Occurring Acetylenes, Academic Press, New York, pp.256-272.

Budzikiewicz, H., Wilson, J.M. and Djerassi, C. (1963). Mass spectrometry in structural and sterochemical problems. XXXII Pentacyclic triterpenes. *J. Amer. Chem. Soc.* **85**: 3688-3699.

Budzikiewicz, H., Djerassi, C. and William, D.H. (1964). Structure Elucidation of Natural Products by Mass Spectrometry, Vol. II. Holden Day Inc., London, p.136-142.

Council of Scientific Industrial Research, India. Publications and Information Directorate, CSIR. Hillside Road, New Delhi, India, Volume X, pp.109-112.

Heywood, V.H. and Harborne, J.B., (eds.) (1977). The Biology and Chemistry of the Compositae, Volume II, Academic Press, London, New York, San Francisco, p.412-430.

Rubinstein, I., Goad, L.J., Clauge, A.D.H. and Mulherin, L.J. (1976). The 220 NMR spectra of phytosterols. *Phytochemistry,* **15**: 195-200.

The Wealth of India (1976). A Dictionary of Indian Raw Materials and Industrial Products. pp.109-112.