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RESEARCH ARTICLE

ISOLATION OF CHEMICAL COMPOUNDS FROM LEAF EXTRACT OF *ALPINIA OFFICINARUM*

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ABSTRACT

This study was undertaken to isolate the chemical compounds from the alcoholic leaf extract of Alpinia Officinarum Hance. Two main chemical compounds, i.e. Alpinia Officinarum (AO-1) Alpinia Officinarum (AO-2), having nomenclature tetradecanyl capriate and bauerenyl arachidate were obtained in the present study by chromatographic methods.

Keywords: *Alpinia Officinarum Hance, Chemical compounds.*

INTRODUCTION

Alpinia officinarum Hance, a perennial herb belonging to family zingiberaceae, is mainly cultivated in Southeast Asia. The plant bears thin and tough rhizomes with orange flesh inside and possesses a sweet-smelling odor and a pungent flavor that have been associated with its spicy flavor and aromatic scent [1-2]. Various phytochemicals such as quercetin, kaemferol, isorhamnetin, galangin, alpinol, and galangol have been reported to be associated with *Alpinia officinarum Hance* [3-5]. Moreover, the ethanolic extract of different parts of the plant has significantly revealed the presence of a range of important bioactive compounds like alkaloids, carbohydrates, glycosides, phenolic compounds, sterols, and acidic compounds [6-8]. However, very less work has been undertaken in order to isolate various chemical compounds form the leaf extract of *Alpinia Officinarum Hance.* Hence, the present study was designed in order to isolate various chemical compounds from the alcoholic leaf extract of *Alpinia Officinarum Hance.*

MATERIALS AND METHODS

1. COLLECTION OF PLANT MATERIAL

The plant material was gifted from AIMIL Pharmaceuticals (I) Ltd., New Delhi. It was authenticated as *Alpinia officinrum hance* at Department of Botany, Jamia Hamdard, New Delhi and a voucher specimen is preserved in the herbarium section of Department of Pharmacognosy, R.I.T., Greater Noida, Uttar Pradesh.

2. PREPARATION OF PLANT MATERIALS

The freshly collected samples were washed and air-dried under shade at room temperature for 7-10 days. After drying, the samples were reduced to small piece, and the material was grounded in to fine powder using pestle mortar, followed by sieving using a muslin cloth. Powdered samples were then stored in air tight containers for further use.

3. EXTRACTION OF PLANT MATERIAL

The plant material which was already air dried, was crushed to smaller pieces, redried, coarsely powdered and was then exhaustively extracted with ethanol (95%) in a Soxhlet Appratus for 72 hours. The extract was filtered and the clear supernatant was collected, covered, labeled and used for the qualitative phytochemical screening.

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4. PREPARATION OF SLURRY

The dried extract of the drug was taken in a china dish and dissolved in minimum quantity of methanol by putting on a water bath. The slurry was made by adding minimum quantity of silica gel (60-120 mesh). It was air dried and finally passed through sieve (No.8) to get uniform particle size.

5. PACKING OF COLUMN

The lower end of a clean dry column was plugged with adsorbent cotton. The column was then half filled with petroleum ether. Silica gel was added in small proportions and allowed to settle down gently until the necessary length of the column was attained. All the air bubbles were allowed to escape by running the column blank thrice with solvent. The dried silica gel slurry of the extract was packed in the column and plugged with the adsorbent cotton and then eluted successively in the order of increasing polarity with different solvents. The development and elution of the column was carried out with successive series of solvents in various combinations, viz., petroleum ether, chloroform in petroleum ether ,chloroform(100%), and methanol in chloroform in increasing polarity.The fractions collected were subjected to thin layer chromatography. Chromatographically identical fractions were combined and concentrated.

ISOLATION OF PHYTOCONSTITUENTS

The following phytoconstituents were isolated (Table 1).

1. Compound AO-1: Elution of the column with petroleum ether-chloroform (4:1) gave crystals of AO-1, recrystallized from acetone-Methanol (1:1), 300 mg (0.08% yield), alongwith R_f value of 0.62 (Petroleum ether-Chloroform, 7:3) and melting point (m.p.) 80-81^oC.

(a) IR υ_{max} (KBr): 2916, 2848, 1738, 1473, 1378, 1174, 1035, 862, 802, 719 cm^{-1}.

(b) ^{1}HNMR ($CDCl_3$): δ 4.08 (1 H, d, J=6.3 Hz, H_2-1'a) 4.03 (^{1}H, d, J=6.9 hz, H_2-1'b), 2.23 (1H, d, J=6.6 Hz, H_2-2a), 2-18 (1H, d, J=7.2 Hz, H_2 -2b), 1.54 (4 H, m 2×CH_2), 0.80 (1 H, t, J=6.6 Hz, Me- 10), 0.78 (3 H, t, J = 6.5 Hz, Me-14').

(c) ^{13}CNMR ($CDCl_3$): δ 173.87 (C-1), 60.09(C-1'), 34.38 (C-2), 31.91 (CH_2) 29.68 (11× CH_2), 29.45 (2×CH_2), 29.35 (CH_2), 29.25 (CH_2), 29.14 (CH_2), 24.97 (CH_2), 22.67 (CH_2), 14.22 (Me-10), 14.08 (Me-14').

(d) +ve TOF MS *m/z* (rel. int.): 368 $[M]^+$, ($C_{24}H_{48}O_2$) (1.1), 213 (21.6), 155 (39.8).

2. Compound AO-2: Elution of the column with petroleum ether-chloroform (7:3) gave crystals of **AO-2** recrystallized from acetone-Methnol (1.1), 370 mg (0.09% yield), alongwith R_f value of 0.63 (Petroleum ether-Chloroform, 6:4) and m.p. 146-147^{0}C.

(a) IR υ_{max} (KBr: 2918, 2849, 1733, 1640, 1462, 1369, 1247, 1025, 961, 875, 719 cm^{-1}.

(b) ^{1}HNMR ($CDCl_3$): Table 2.

(c) ^{13}CNMR ($CDCl_3$): Table 2.

(d) +ve TOF MS *m/z* (rel. int.): 718 $[M]^+$, ($C_{50}H_{86}O_2$) (1.3), 312 (18.3), 302 (23.2), 295 (15.2), 274 (22.6), 232 (19.2).

RESULT AND DISCUSSION

Compound AO-1 named Tetradecanyl capriate, was obtained as colourless crystalline mass from petroleum ether-chloroform (4:1) eluents. It did not decolorize bromine water, which suggests the saturated nature of the molecule. Its IR spectrum showed characteristic absorption bands for ester group (1738 cm^{-1}) and long aliphatic chains (802, 719 cm^{-1}). Further, the mass spectrum of AO-1 exhibited molecular ion peaks at *m/z* 368 corresponding to the molecular formula of a fatty acid ester, $C_{24}H_{48}O_2$. It indicated one double bond equivalent which was adjusted in the ester function. The generation of the ion fragment at *m/z* 155 $[CH_3(CH_2)_8CO]^+$ and 213 $[O(CH_2)_{13}CH_3]^+$ indicated that capric acid was esterified with a C_{14} alcohol.

Moreover, the ^{1}HNMR spectrum of AO-1 exhibited four proton doublets at δ 4.08 (J=6.3 Hz) and 4.03 (J=6.9 Hz) assigned at onto oxygenated methylene H_2-1' proton and at δ 2.23 (J=6.6Hz) and 2.18 (J=7.2 Hz), which ascribed to the methylene H_2-2 proton adjacent to the ester groups. Three proton triplets at δ 0.80 (J=6.6 Hz) and 0.78 (J=6.5 Hz) were ascribed to primary C-10 and C-14' methyl proton, respectively. The remaining methylene proton appeared as the multiplets δ 1.54 (4 H) and 0.96 (2 H) and broad signal at δ 1.18 (32 H).

The ^{13}CNMRspectrum of AO-1 displayed signals for the ester carbon at δ 60.09 (C-1') and other methylene carbons between δ 34.38-22.67 and methyl carbons at δ 14.22 (C-10) and 24.97 (C-14'). The absence of any signal beyond δ 4.08 in the ^{1}HNMR spectrum between 173.87-60.09 in the ^{13}C NMR spectrum supported the saturated nature of the molecule. The alkaline hydrolysis of AO-1 yielded capric acid, Co-TLC comparable on the basis of the spectral data analysis and chemical reactions. The structure of AO-1 has been identified as *n*-tetradecanyl n-decanvate.

$CH_2\ (CH_2)_{18}CO\text{-}O\ (CH_2)_{13}CH_3$
Chemical structure of Tetradecanyl capriate (AO-1)

$CH_3\ (CH_2)_8CO\text{-}O\ (CH_2)_{13}CH_3$
m/z 368 (1.1)

$[CH_3\ (CH_2)_8CO]^+$
m/z 155 (39.8)

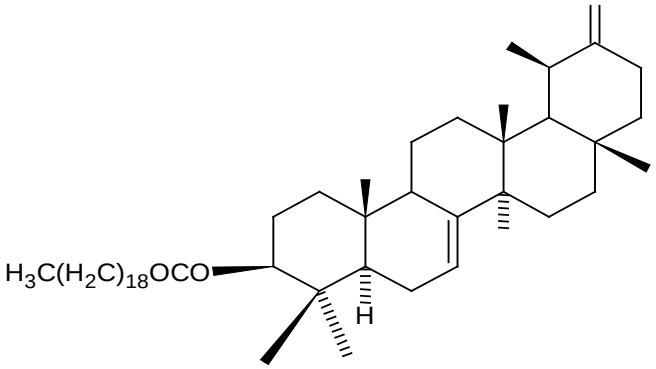
$[OCH_3\ (CH_2)_8CO]^+$
m/z 213 (21.6)

Scheme - 1. Mass fragmentation pattern of Tetradecanyl capriate (AO-1).

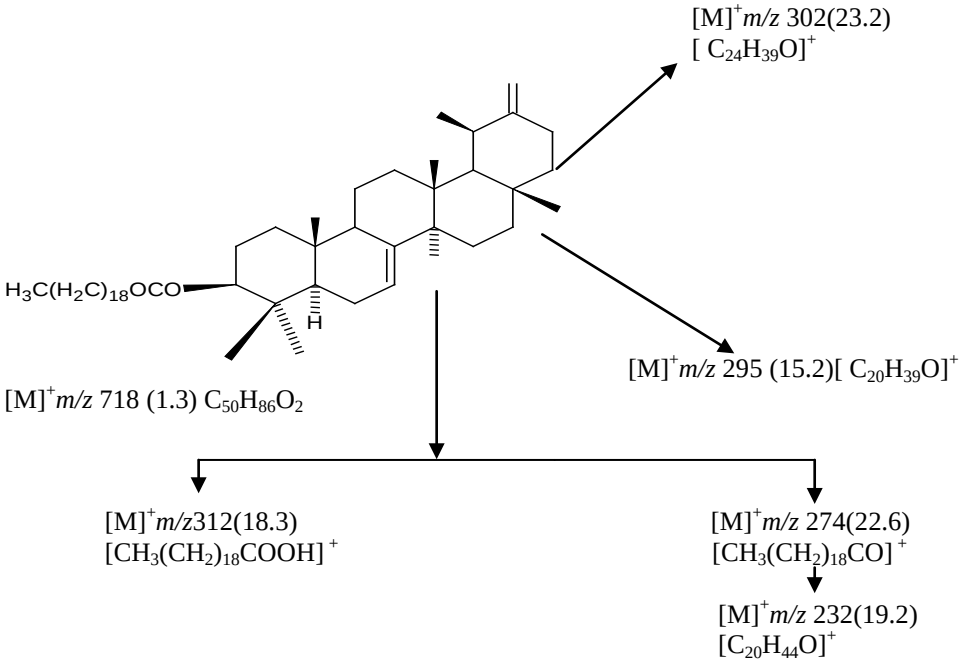
Compound AO-2 named Bauerenyl arachidate, was obtained as a colourless cryatalline mass from petroleum ether-chloroform (7.3) eluents. It responded positively to Liebermann-Burchamardt test for the presence of triterpenoids. Its IR spectrum showed characteristic absorption bands for ester groups (1633 cm^{-1}), unsaturation (1640), and long aliphatic chains (875, 719 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, its molecular weight was established at *m/z* 718 corresponding to the pentacyclic triterpenoid esrer, $C_{50}H_{86}O_2$. The generation of the ion fragments at *m/z* 312 $[CH_3\ (CH_2)_{18}COOH]^+$and 295 $[CH_3(CH_2)_{18}CO]^+$ indicated that arachidonic acid was esterified with the triterphenol. The formation of the ion peaks at *m/z* 232 $[C_{8,14}\text{-}C_{9,11}$ fission$]^+$ and 274 $[(C_{14'15}\text{-}C_{13'18}$ fission$]^+$ supported the location of one of the linkages in ring B. The molecular ion peak showed eight double bond equivalents, in which five of them were adjusted in the pentacyclic carbon frame work of the molecule, two in the vinylic linkages and the remaining one in the ester groups.

The ^{1}HNMR spectrum of AO-2 displayed one proton doublet at δ 5.27 assigned to vinylic H-7 proton. Two single proton broad signals at δ 5.01 and 4.95 were ascribed to exocyclic C-30 methylene proton. A one-proton double doublet at δ 4.48 with interaction of 5.5, 9.8 Hz were attributed to carbinol H-3α proton and its deshielding location, which indicated the presence of the ester groups at this carbon. Six three-proton broad signals at δ 1.12, 1.04, 0.85, 0.73, 1.02 and 0.97 were associated with the C-23, C-24, C-25, C-26, C-27 and C-28 primary methyl protons respectively. A three proton double at δ 0.87 (J=7.8Hz) and a three proton triplet at δ 0.85 (J=6.5 Hz) were accounted with C-29 secondary and C-20' primary methyl proton. A two-proton doublet at δ 2.39 (J=15.8 Hz) was due to C-18 methylene proton. The remaining methane and methylene proton resonated in the range of δ 2.61-0.94. Also, the appearance of all the methyl signals in the range of δ 1.12-0.85 indicated that all these functionalities were located on the saturated carbon.

The ^{13}CNMR spectrum of AO-2 exhibited signals for ester carbon at δ 118.88 (C-7), 139.82 (C-8), 154.64 (C-20) and 107.11 (C-30), oxygenated methane carbon at δ 80.96 (C-30 and methyl carbons between δ 33.15-14.17. The ^{1}H and ^{13}CNMR values of AO-2 were compared with bauerenyl ester type molecules of already reported studies. Furthermore, the alkaline hydrolysis of AO-2 yielded arachidonic acid, TLC-comparable, and bauerdienol. On the basis of spectral data analysis and chemical reactions the structure of AO-2 has been formulated as bauere-7, 20(30)-dien-3β-oyl eicosanoate, which is a new pentacyclic triterpenoid isolated from a natural source for the first time.



Chemical structure of bauerenyl arachidate (AO-2).



Scheme - 2. Mass fragmentation pattern of bauerenyl arachidate (AO-2).

Code	Name	Column eluant	R_f value mobile phase	Yield (% w/w) solvents used for recrystallization	m.p. °C	Mol. wt. [mol. for.]	Nomenclature
AO-1	Tetradec-anyl capriate,	4:1 P:C	.62 P:C 7:3	0.08 (A:M) 1:1	88	$C_{24}H_{48}O$ 368	Trtradecanyl capriate
AO-2	Baueren-yl arachidate	7:3 P:C	.63 P:C 6:4	0.09 (A:M) 1:1	146-147	$C_{50}H_{86}O_2$ 718	Bauerenyl arachidate

Table - 1. Chemical constituents isolated from the alcoholic leaf extract of *Alpinia Officinarum Hance.*
A, Acetone; C, Chloroform; M, Methanol; P, Petroleum Ether; EtoAc, ethyl acetate

Position	^{1}H NMR		^{13}C NMR	Position	^{1}H NMR		^{13}C NMR
	Alpha	Beta			Alpha	Beta	
1.	1.78 m	2.04	39.18	26	0.73brs	-	17.69
2	2.41m	2.25	27.03	27	1.02brs	-	21.61
3	4.48dd	5.55,9.8	80.96	28	1.02brs	-	33.15
4	-	-	38.43	29	0.87d(7.8)	-	26.14
5	0.94m	-	50.34	30	5.01brs	4.95brs	107.11
6	2.24m	1.68m	25.60	1’	-	-	42.20
7	5.27d(5.2)	-	118.88	2’	2.04brs	-	35.15
8	-	-	139.82	3’	1.55	-	29.6
9	2.19m	-	48.67	4’	1.25	-	29.68
10	-	-	37.79	5’	1.25	-	29.68
11	1.39m	1.61	23.69	6’	1.25	-	29.68
12	1.30	1.47m	36.31	7’	1.25	-	29.68
13	-	-	37.75	8’	1.25	-	29.68
14	-	-	42.16	9’	1.25	-	29.68
15	1.70m	1.41m	31.92	10’	1.25	-	29.68
16	-	-	31.92	11’	1.25brs	-	29.51
17	-	-	34.16	12’	1.25brs	-	29.51
18	2.39d(15.5)	-	55.40	13’	1.25brs	-	29.51
19	2.61m	-	36.69	14’	1.25brs	-	29.51
20	-	-	154.64	15’	1.25bra	-	29.45
21	2.43m	2.24m	40.19	16’	1.25brs	-	26.95

22	2.57m	1.58m	38.86	17’	1.25brs	-	26.95
23	1.04 brs	-	16.49	18’	1.25brs	-	25.57
24	0.85 brs	-	18.18	19’	1.21brs	-	22.68
25	0.73 brs	-	19.47	20’	0.85t(6.5)	-	14.17

Table - 2. ^{1}HNMR and ^{13}CNMR spectral data of Bauerenyl arachidate (AO-2).

CONCLUSION

Two chemical compounds with nomenclature, tetradecanyl capriate and bauerenyl arachidate were isolated form the alcoholic leaf extract of *Alpinia Officinarum Hance*. Although, the present study has aimed to isolate various chemical compounds from the leaves of *Alpinia Officinarum Hance*, but further studies are needed completely isolate the chemical compounds form the plant extract..

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