CHAPTER IV

Structures of Esters A, B and C

STRUCTURES OF ESTERS A,B AND C

In the preceeding two chapters, isolation and structure determination of jaeschkeanatriol, epoxyjaeschkeanadiol and neojaeschkeanadiol have been described. These sesquiterpene alcohols were isolated from the neutral portion of the saponified total acetone extracts of Ferula jeschkeana Vatke roots. Since, the TLC of the total acetone extract did not show the presence of these compounds in any significant amount, it was quite reasonable to assume that these alcohols must be occuring as esters in their natural form. It was therefore of interest to isolate these esters. With a view to isolating these esters, a systematic chromatography was undertaken and as a result three esters have been isolated. These esters have been designated as esters A, B and C. The isolation of these esters has been described in the experimental section of this chapter.

Structure of ester A

Compound A, m.p. 84-85° $[\alpha]_D$-1.98°(c, 1.51) analyses for $C_{27}H_{36}O_6$ and has in its mass spectrum (Fig.I)M^+-H_2O at m/e 438. Its IR(Fig.II) shows bands due to OH(3350 cm^{-1}), C=O(1680 cm^{-1}) and C=CH(aromatic),(1600 cm^{-1}). Its UV shows λ max 219 nm(ϵ,12860) and 261 nm(ϵ,17930). The PMR(Fig. III) of the compound shows presence of $-C-\underline{Me}$ (3H, s, 1.16 ppm),$-CH\underline{Me}_2$(6H, two doublets centered at

150

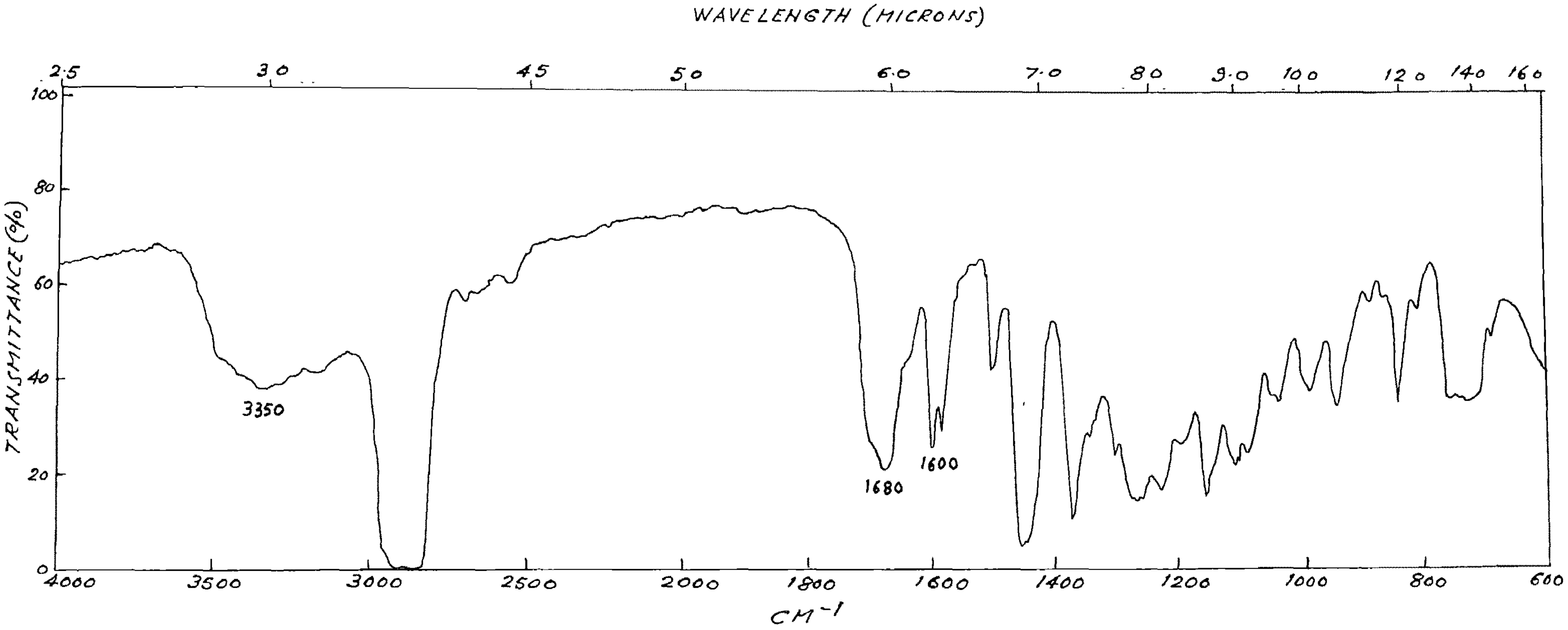

FIG. II. IR SPECTRUM OF ESTER A

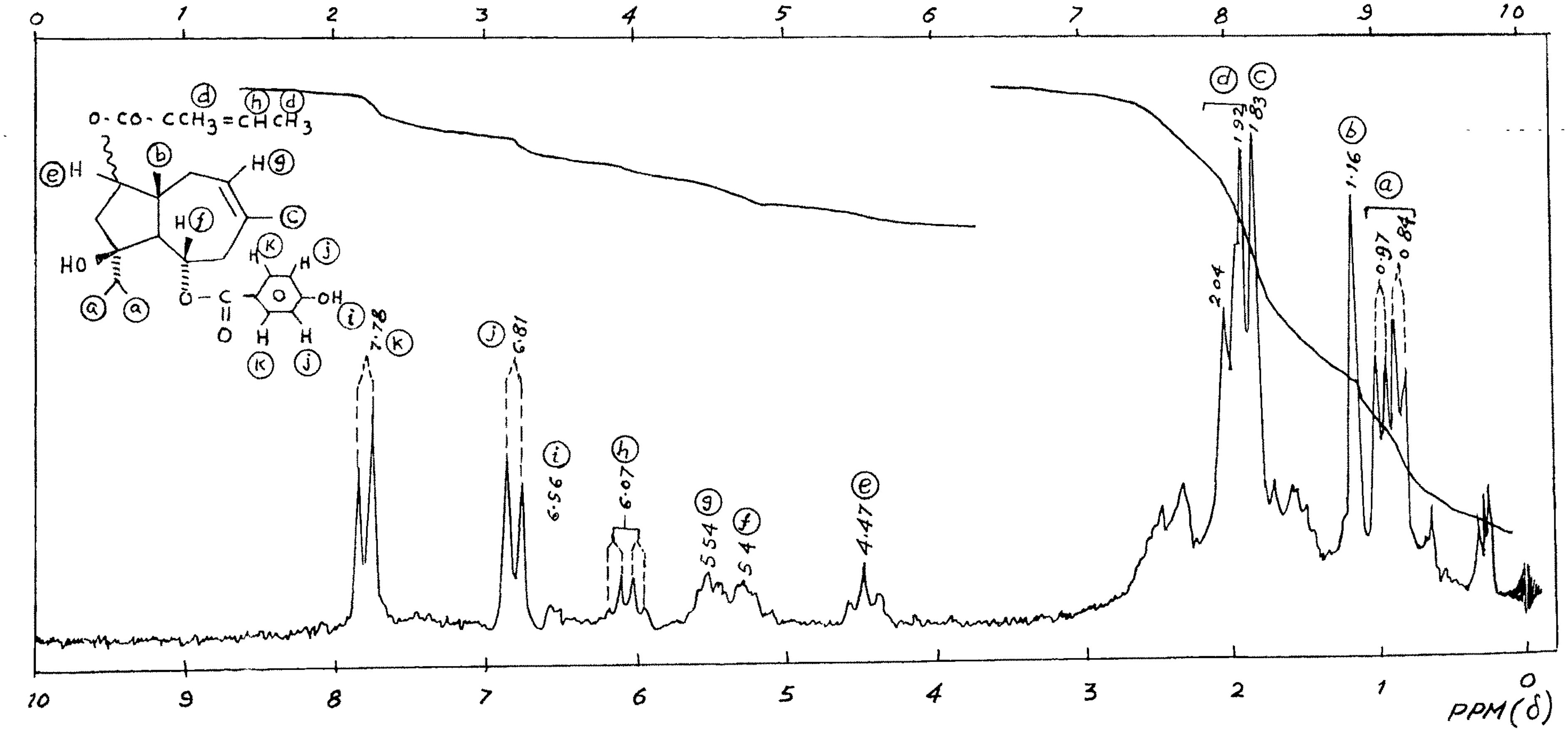

FIG. III. PMR SPECTRUM OF ESTER A

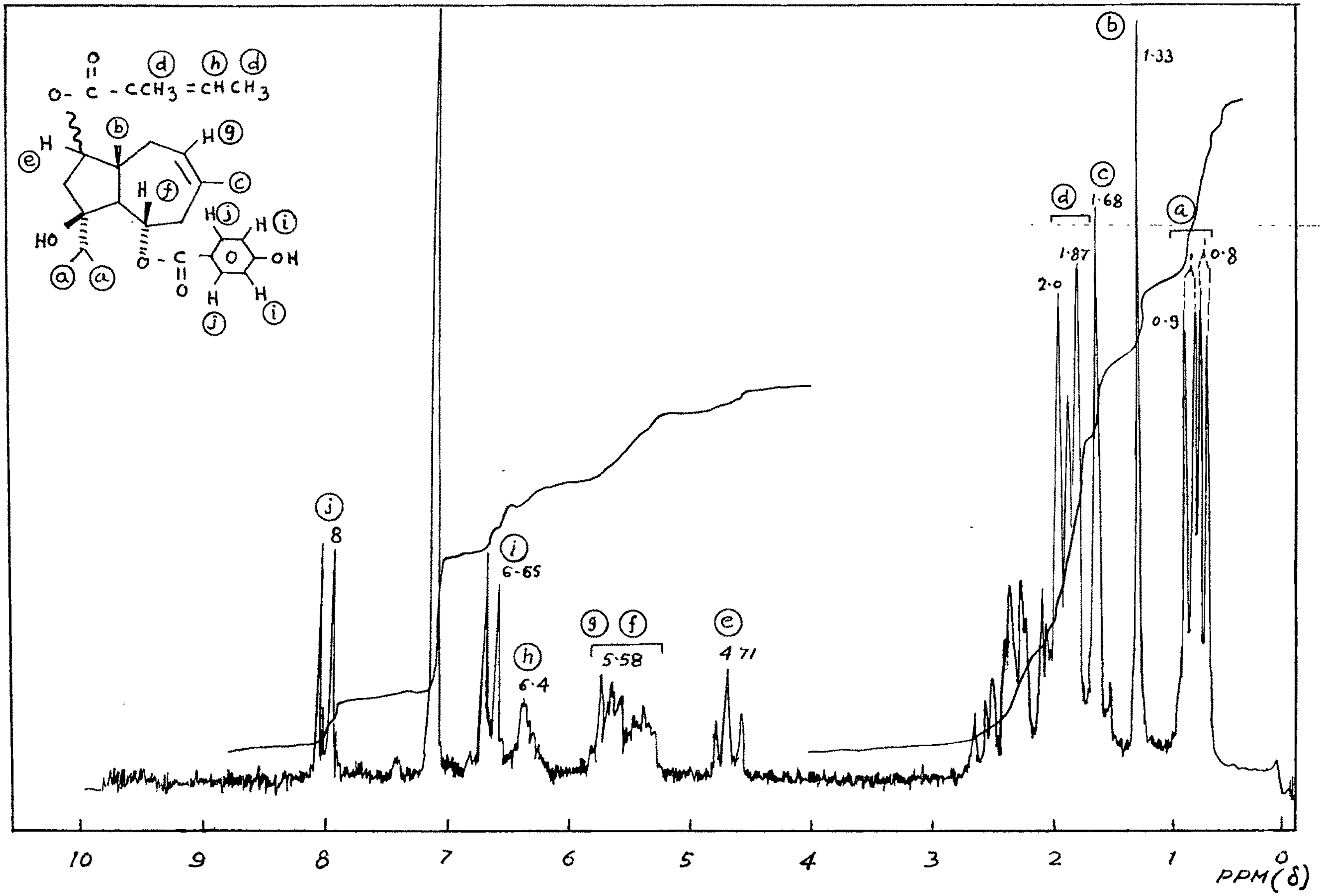

FIG. IV. PMR SPECTRUM OF ESTER A (IN C_6D_6)

0.84 and 0.97 ppm, J=7Hz each),$-C=C\underline{M}e$(3H, s, 1.83 ppm), $-COC\underline{M}e= CH\underline{M}e$96H, m, between 1.92 and 2.04 ppm), two $C\underline{H}-O-CO-C$(1H each, m, at 4.47 and 5.4 ppm),$-C=C\underline{H}$ (1H, m, 5.54 ppm), $-COC\underline{M}e=CH\underline{M}e$(1H, q, centered at 6.07 ppm, J=7Hz), Ar=OH(1H, s, 6.56 ppm) and $Ar\underline{H}$(4H, a typical AA'BB' quartet of p,p'-disubstituted benzene type centered at 7.27 ppm). By D_2O exchange, the presence of two hydroxyls is inferred. Based on these spectral data it can be reasonably concluded that the compound A is an ester of jaeschkeanatriol.

Compound A was hydrolysed using 10% methanolic KOH under refluxing conditions. The neutral compound obtained from the hydrolysis was found to be jaeschkeanatriol (m.p., mixed m.p., PMR). The acidic fraction was methylated using methanol-sulfuric acid. The GLC(10% silicone SE 30; temp 160°) of the total esters indicated it to be a mixture of two compounds. The major compound (RT 7.2; 82%) was found to be p-hydroxy methylbenzoate and the minor compound (RT 0.4; 18%) was identified as methyltiglate by coinjection with authentic samples. Thus ester A is a diester based on jaeschkeanatriol, p.hydroxybenzoic acid and tiglic or angelic acid*.

As mentioned earlier, the mass spectrum of compound A shows M^+-H_2O at m/e 438. The other prominent peaks are at m/e 218,175,121,83 and 55. The fragments at m/e 83 and 55 are typical of cleavage of esters of angelic

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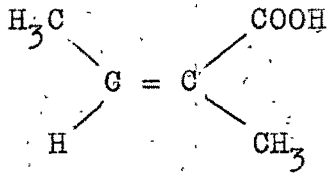
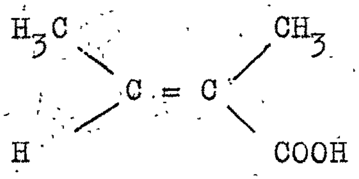
*Angelic acid is known to isomerise to tiglic acid under acidic conditions[3] as well as alkaline conditions[5].

 and tiglic 

acids[1]. The presence of multiplet at 6.07 ppm in the PMR of compound A indicates a typical chemical shift of the vinyl proton of angelic acid, whereas the vinyl proton of tiglic acid appears at 7.0 ppm[2]. Furthermore, the PMR(Fig.IV) of compound A in C_6D_6 shows vinyl proton at 6.4 ppm(δ CCl_4- C_6D_6=0.33 ppm), thus establishing the trans relationship of the vinyl proton to the ester function[3,4]. So of the two acids esterifying jaeschkeanatriol, one is angelic acid, the other being p-hydroxybenzoic acid.

Location of the two ester groups on jaeschkeanatriol: Having established the identity of the two esterifying acids, the next problem was to determine their site of attachment on jaeschkeanatriol. This was conveniently sorted out from the results of the partial hydrolysis of ester A. Compound A was partially hydrolysed using 5% KOH in aqueous dioxane (15 hr). After the work up, two compounds (one, of the mebing jaeschkenatriol) were isolated pure from the neutral protion by chromatography over SiO_2-gel. The faster moving compound (Rf 0.47), b.p. 200-210° (bath)/0.1 mm analyses for $C_{20}H_{32}O_4$(M^+-H_2O at m/e 318) and shows presence of OH(3590, 3530, 1040 cm^{-1}); C=O(1705, 1160 cm^{-1}) and C=CH(1640 cm^{-1}) in its IR (Fig. VI) spectrum. Its PMR (Fig.VII) shows following structural features -C-$\underline{Me}$(3H, s, 1.1 ppm),-CH$\underline{Me}_2$(6H, two

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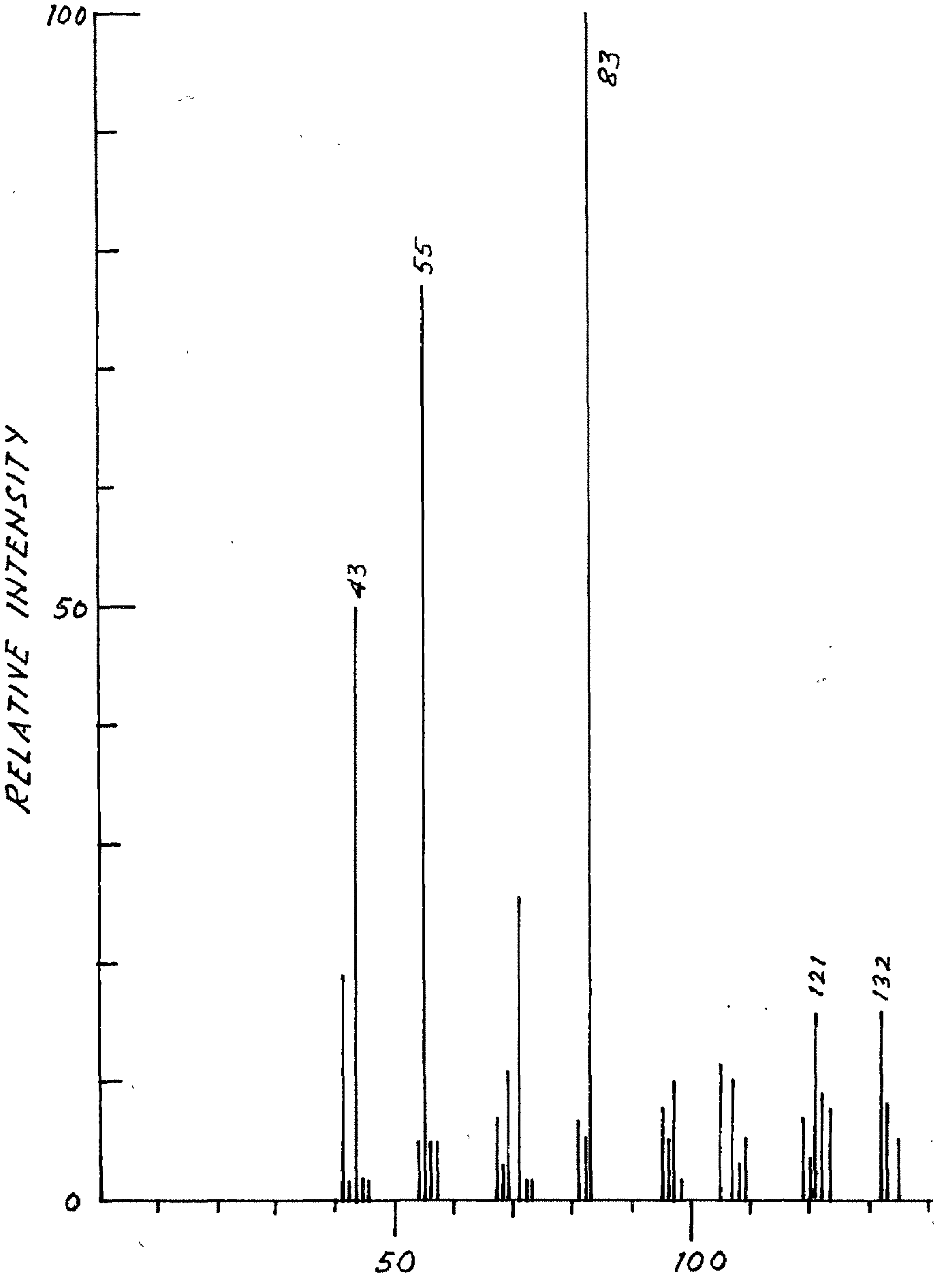


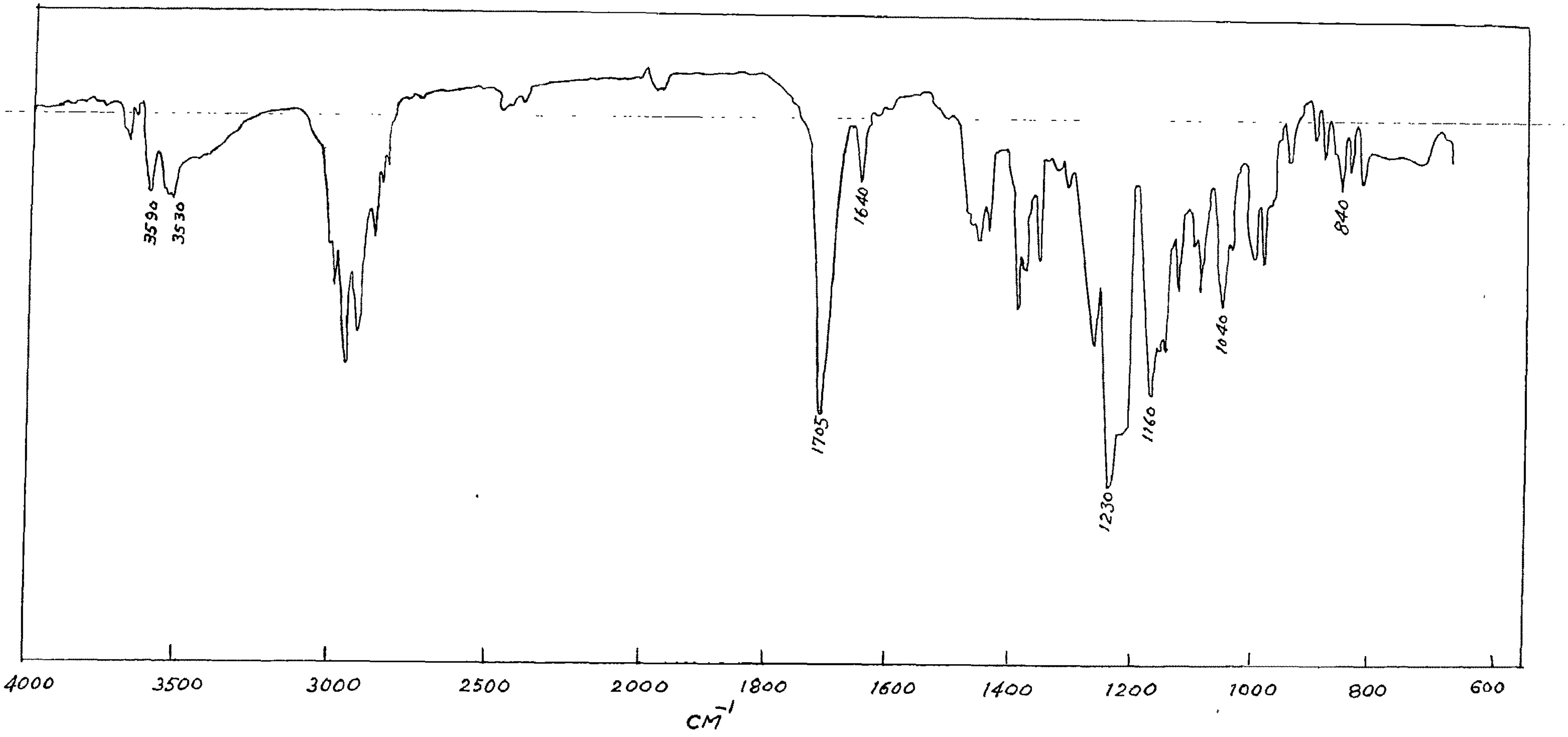


FIG. VI. IR SPECTRUM OF THE PRODUCT FROM THE PARTIAL HYDROLYSIS OF ESTER A

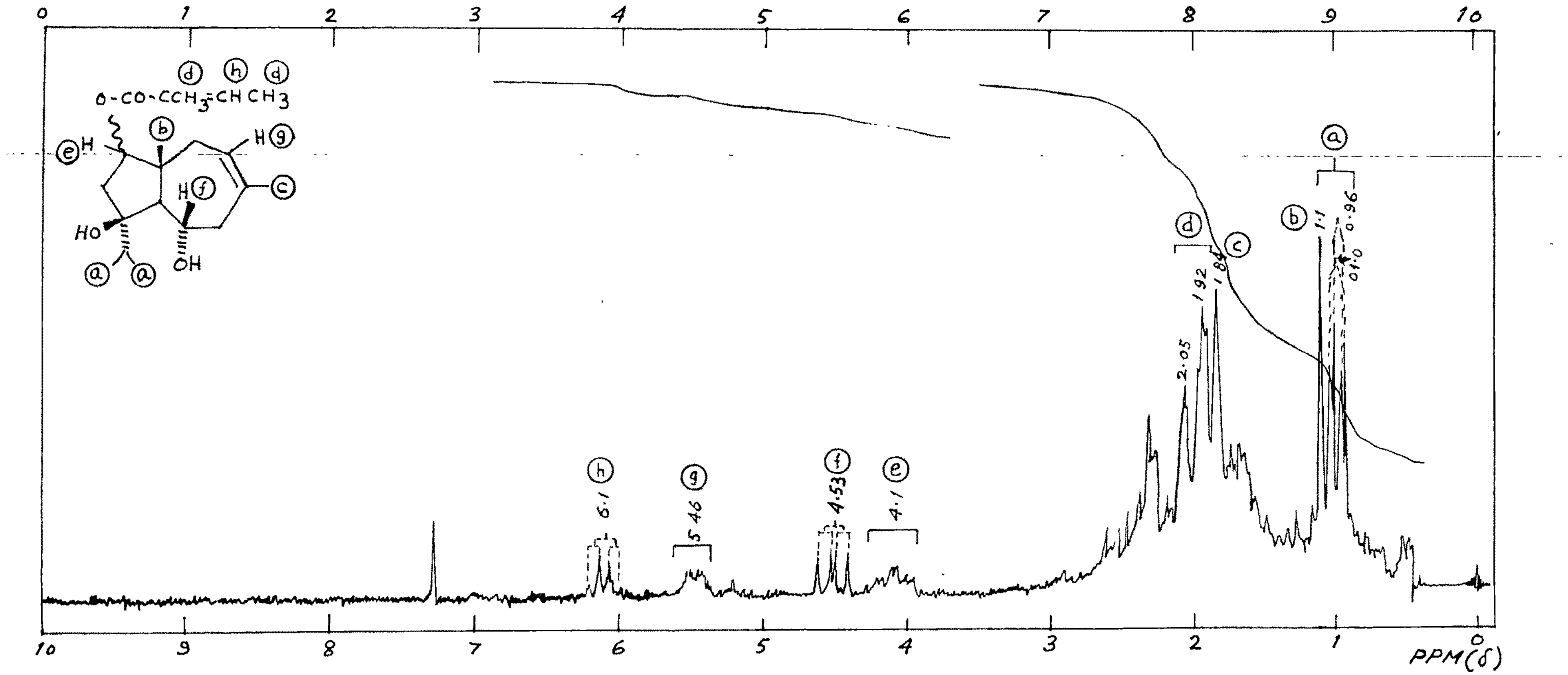
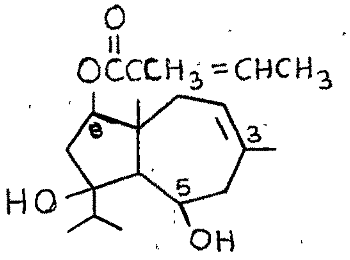


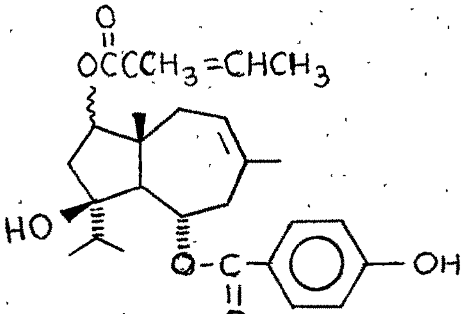
FIG. VII. PMR SPECTRUM OF THE PRODUCT FROM THE PARTIAL HYDROLYSIS OF ESTER A doublets centered at 0.96 and 1 ppm, J=7 Hz each), $-C{=}C\underline{Me}$(3H,s,1.84 ppm),$-CO-C\underline{Me}{=}CH\underline{Me}$(6H, m, between 1.92 and 2.05 ppm), one $C\underline{H}OH$(1H, m, 4.1 ppm),$-CH-O-CO-$(1H, two doublets centered at 4.53 ppm, J_1=3Hz and J_2=8Hz)$-C{=}C\underline{H}$ (1H, m, 5.46 ppm) and $-COCMe{=}C\underline{H}Me$(1H, q, centered at 6.1 ppm, J=7 Hz). Thus from these data it is quite evident that the ester cleavage resulted into the loss of p-hydroxybenzoic acid moiety.

In the PMR of jaeshkeanatriol (Chapter II), the C_5 proton appears at 4.1 ppm and C_8 proton at 3.42 ppm. Since in the PMR of the partially hydrolysed product (Rf 0.47), the $C\underline{H}OH$ signal appears at 4.1 ppm(an upfield shift of 1.3 ppm as compared to its signal in compound A), it is quite clear that the hydroxyl at C_5 is esterified by p-hydroxybenzoic acid. Thus the partially hydrolysed product could be represented by the following structure (I)



(I)

Compound A, would then in turn be represented by structure (2)



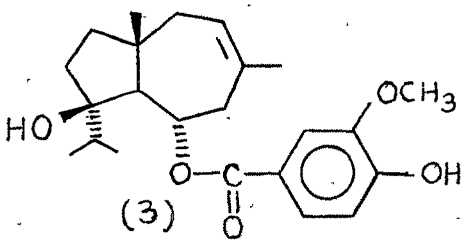
(2)

Structure of ester B

Compound B b.p. 210–220° (bath)/0.2 mm $[\alpha]_D$+40.12° (c, 1.62), analyses for $C_{23}H_{32}O_5$ and has in its mass spectrum (Fig. VIII) the M^+– 43 at m/e 345. Its IR(Fig.IX) shows absorptions due to OH(3440, 1030 cm^{-1}),C=0(1685 cm^{-1}) and C=CH(aromatic)(1590 cm^{-1}). Its UV has λ max ч+226 nm (∈, 11,190), 269 nm(∈,8468) and 297 nm (∈,3847). The PMR (Fig.X) of the compound B reveals the following structural features:-C-<u>Me</u>(3H,s,1.12 ppm),-CH<u>Me</u>$_2$(6H, two doublets centered at 0.86 and 0.97 ppm, J=7Hz each),-C=C<u>Me</u>(3H, s, 1.85 ppm), Ar-OMe(3H, s,3.97 ppm),-<u>CH</u>-O-CO-C and -C=C<u>H</u> (2H, bm, at 5.36 ppm), Ar-OH (1H,s,6.1 ppm) and Ar<u>H</u> (3H, m, between 6.92 and 7.67 ppm)

Compound B on hydrolysis with alcoholic KOH gave a neutral compound m.p. 91–92° which was identified as jaeshkeanadiol (mixed m.p.IR,PMR) The acidic component, m.p. 205–206° obtained from this hydrolysis was converted into its methylester using diazomethane. It was established to be methylester of vanillic acid (IR, PMR,GLC).

Based on the above spectral and chemical data, structure (3) is assigned to compound B*



(3)

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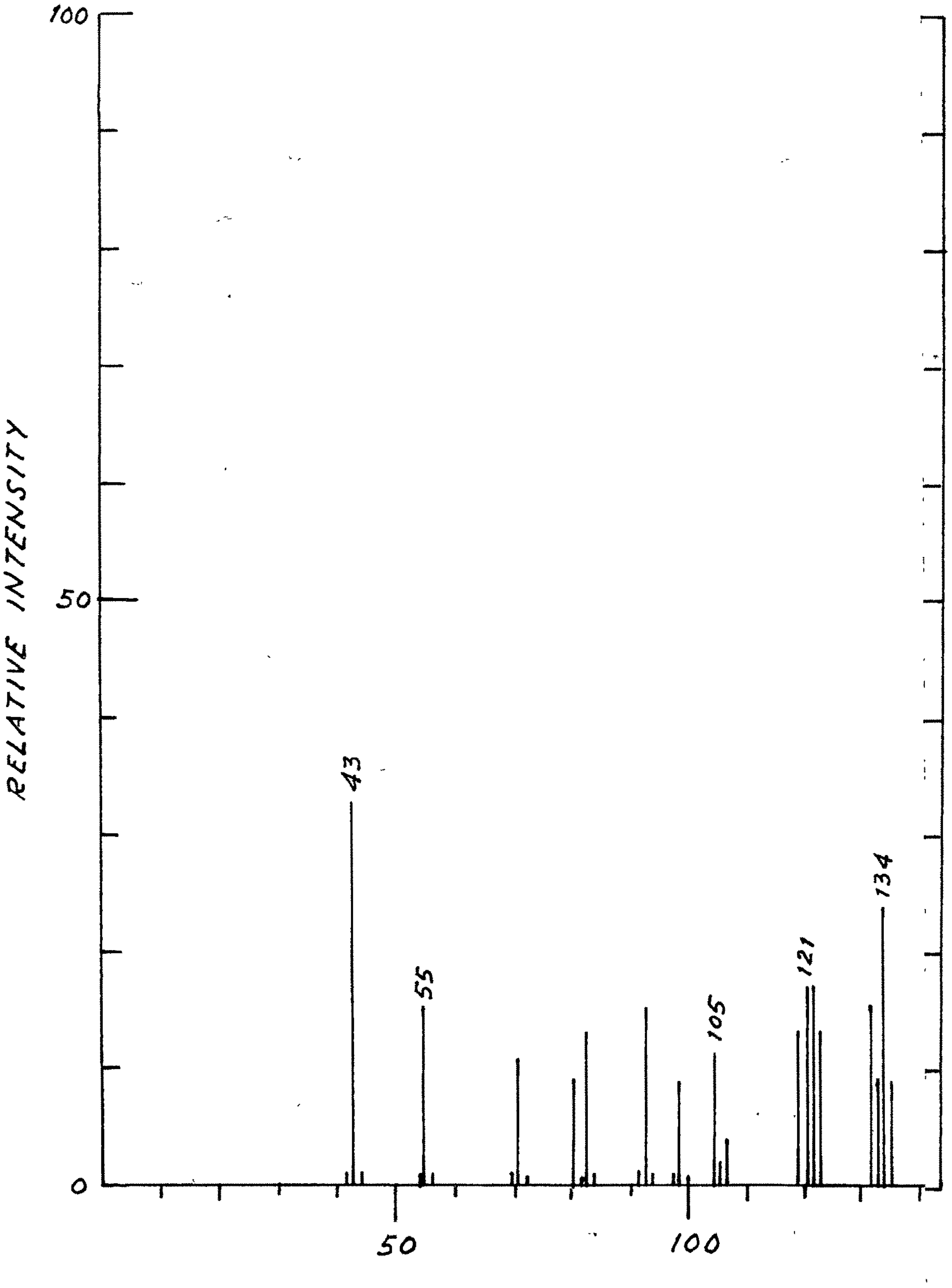
*While this work was under progress, Russian workers reported the isolation of teferin[6] and akitschenin[7] from Ferula tenuisecta and Ferula akitschensis, teferin corresponds to ester B and akitschenin corresponds to ester A.


100
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RELATIVE INTENSITY
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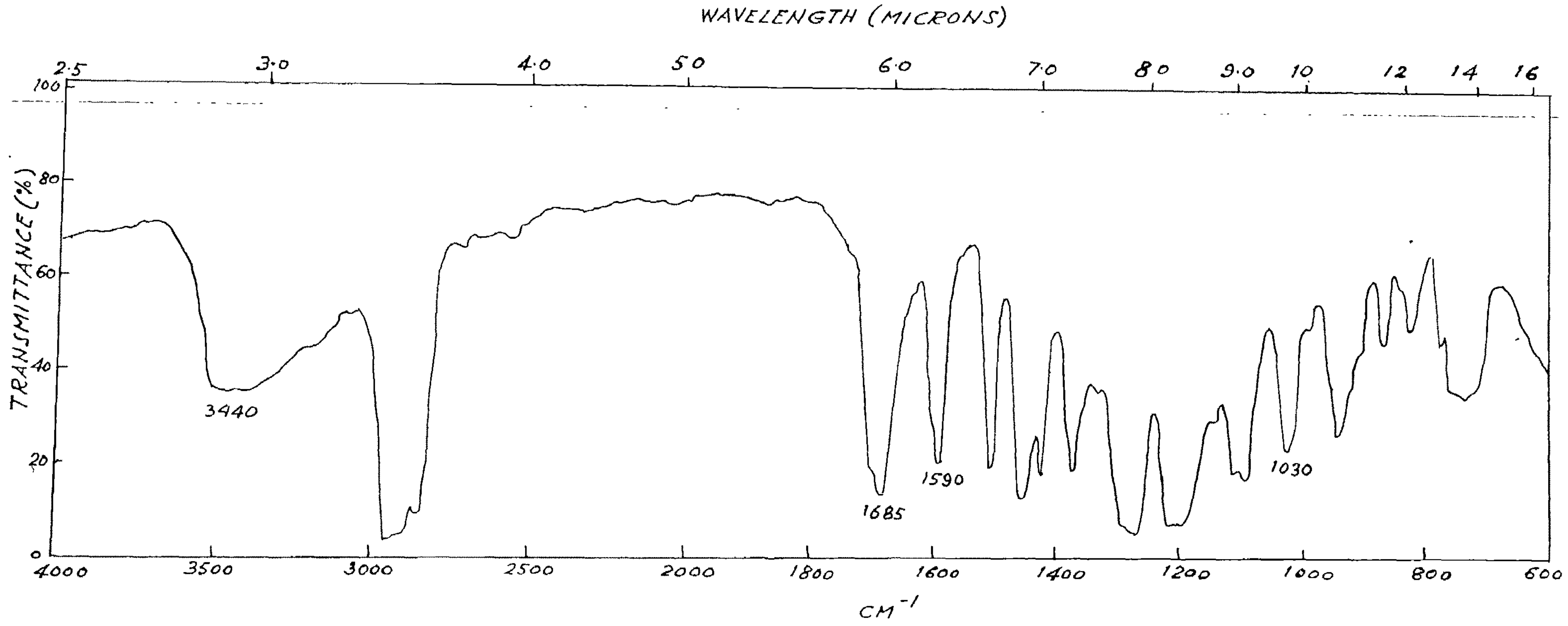


FIG. IX. IR SPECTRUM OF ESTER B

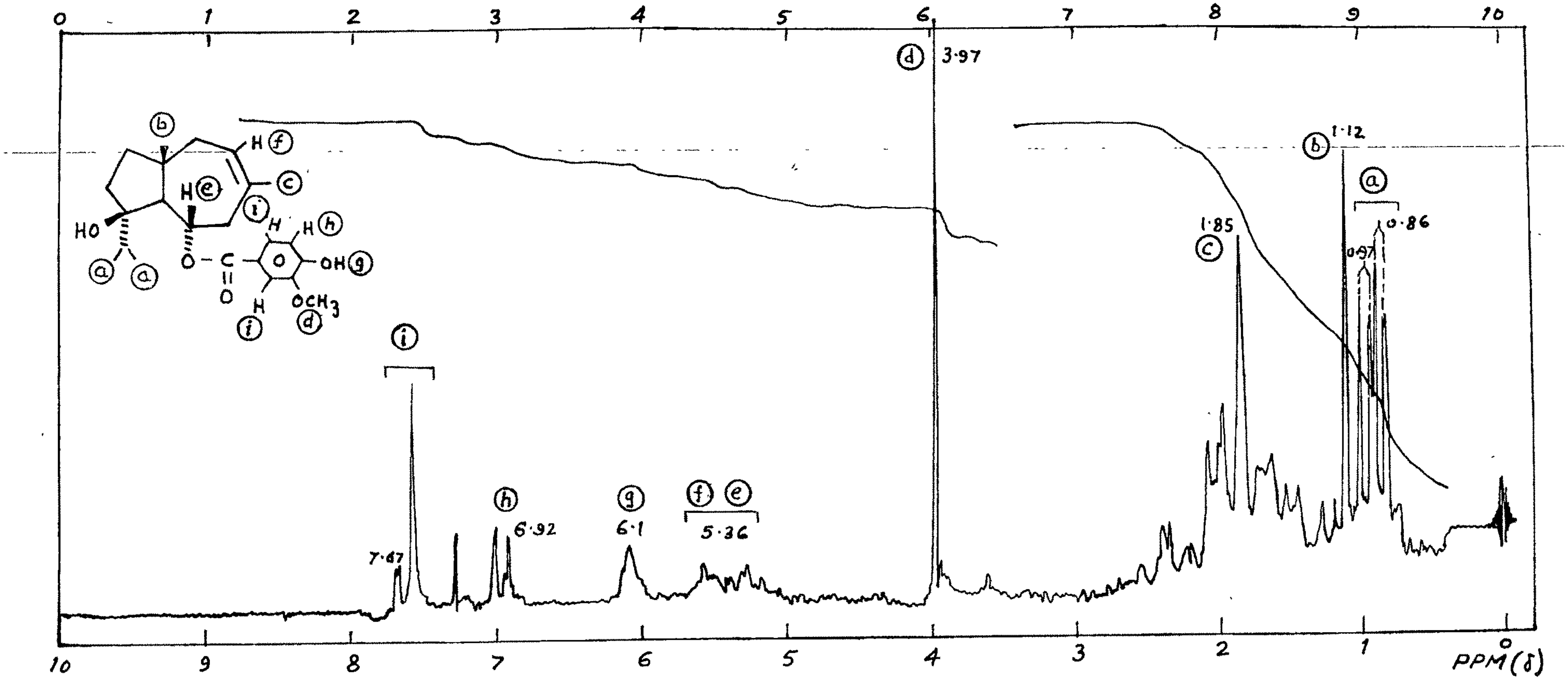


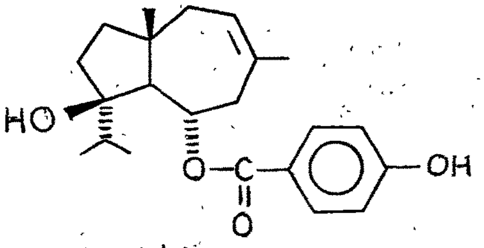
FIG. X. PMR SPECTRUM OF ESTER B

Structure of ester C

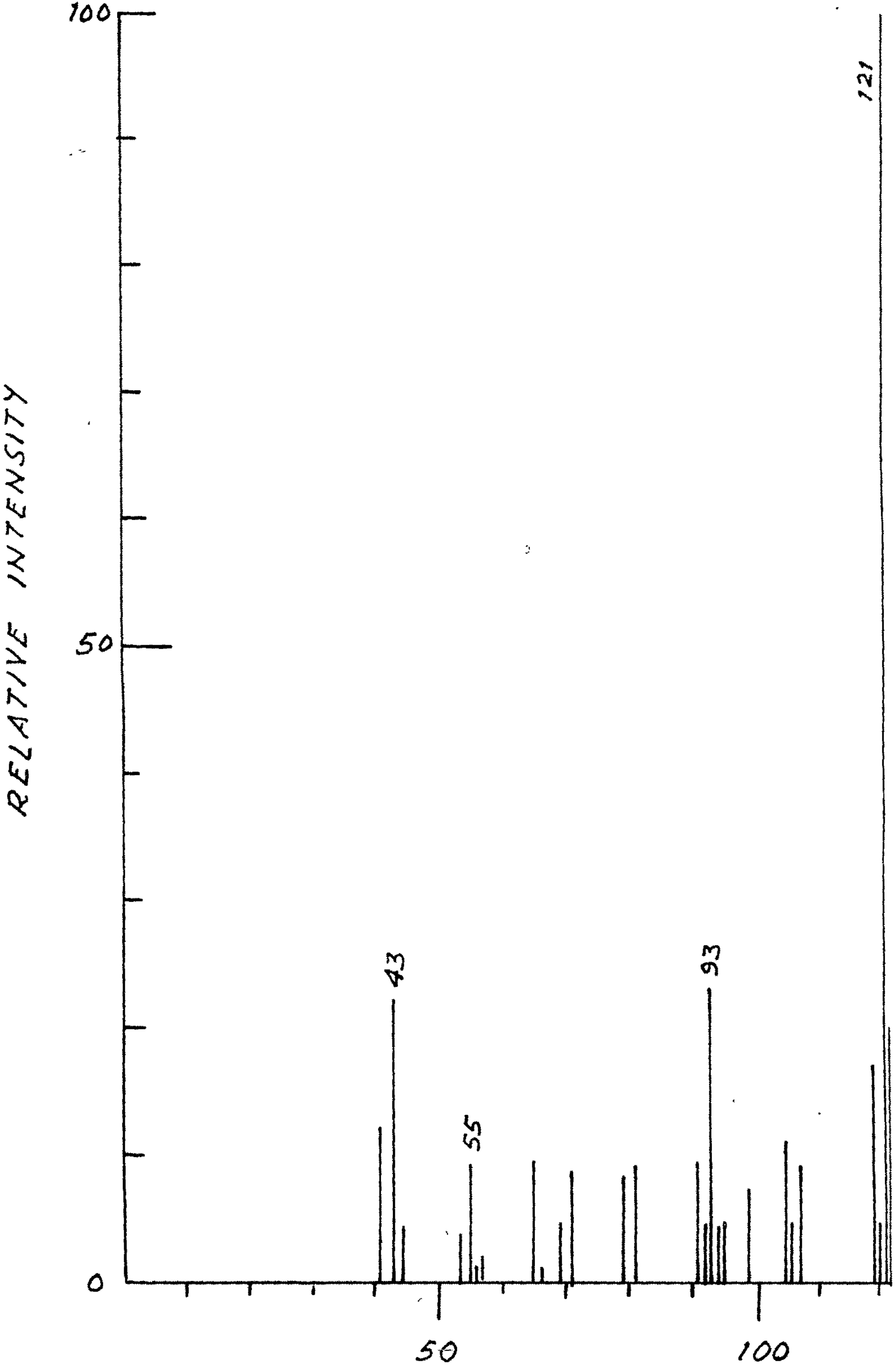
Compound C, b.p. 215-225°(bath)/0.1 mm $[\alpha]_D$+37.76° (c, 1.43) analyses for $C_{22}H_{30}O_4$(M^+-H_2O at m/e 340, Fig.XI). Its UV has max at 214 nm(∈, 7413) and 262 nm (∈, 14,190). Its IR (Fig.XII) shows presence of OH (3300, 1155, 1030 cm^{-1}), C=O(1670 cm^{-1}) and C=CH (aromatic)(1600, 840 cm^{-1}). Its PMR (Fig.XIII) reveals following structural characteristics :-C-Me(3H, s, 1.1 ppm), -CHMe$_2$(6H, two doublets, centered at 0.85 and 0.93 ppm, J=7Hz each), -C=CMe(3H, s, 1.83 ppm),-CH-O-CO-C(1H, triplet of doublets, centered at 5.3 ppm, $J_1=J_3$=3Hz and J_2=10 Hz),-C=CH(1H, m, 5.6 ppm) and ArH (4H, a typical AA'BB' 'quartet' centered at 7.39 ppm).

Compound C was hydrolysed using methanolic KOH. The neutral fraction obtained was established to be jaeshkeanadiol (m.p., mixed m.p., IR, PMR). The acidic component, m.p. 210-211° was transformed into the corresponding methyl ester (CH_2N_2). From its spectral data (IR, PMR), this ester was found to be p-hydroxy methylbenzoate.

On the basis of the above spectral and chemical data, structure (4) is suggested for compound C.



(4)


RELATIVE INTENSITY
100
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121
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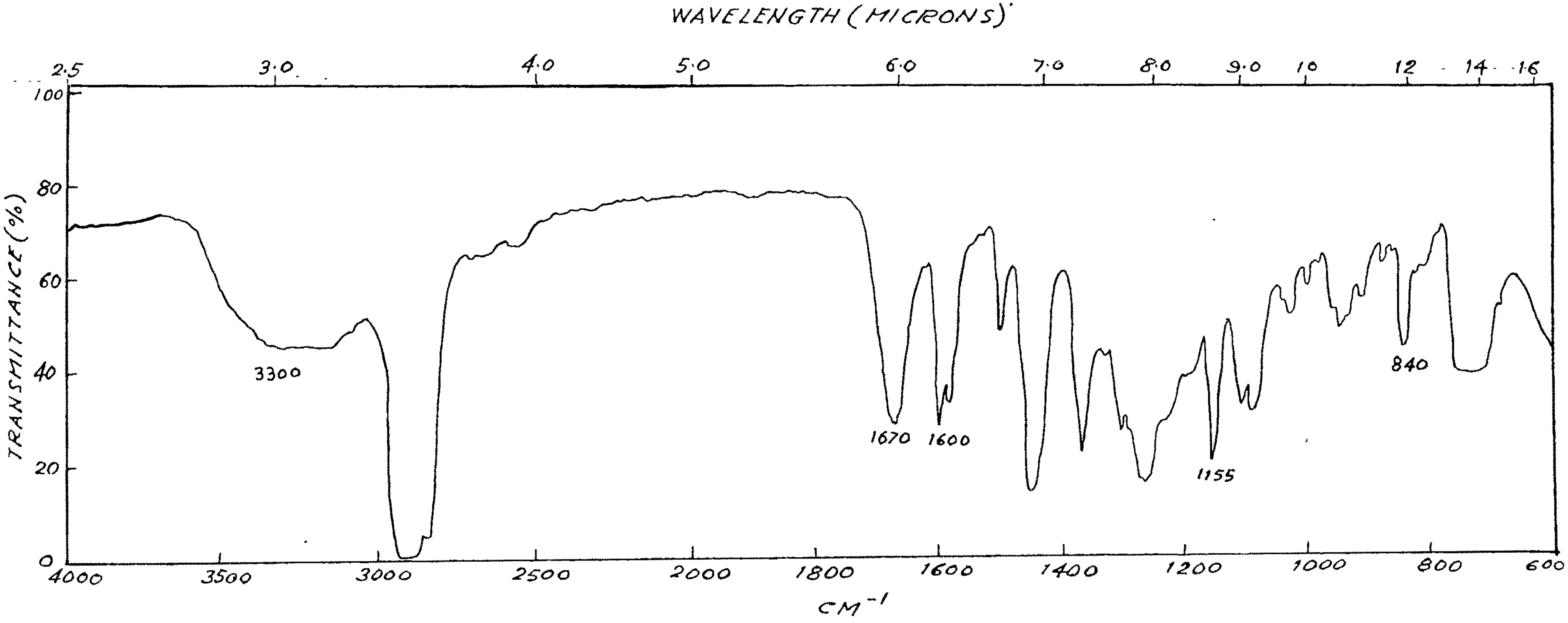


FIG. XII. IR SPECTRUM OF ESTER C

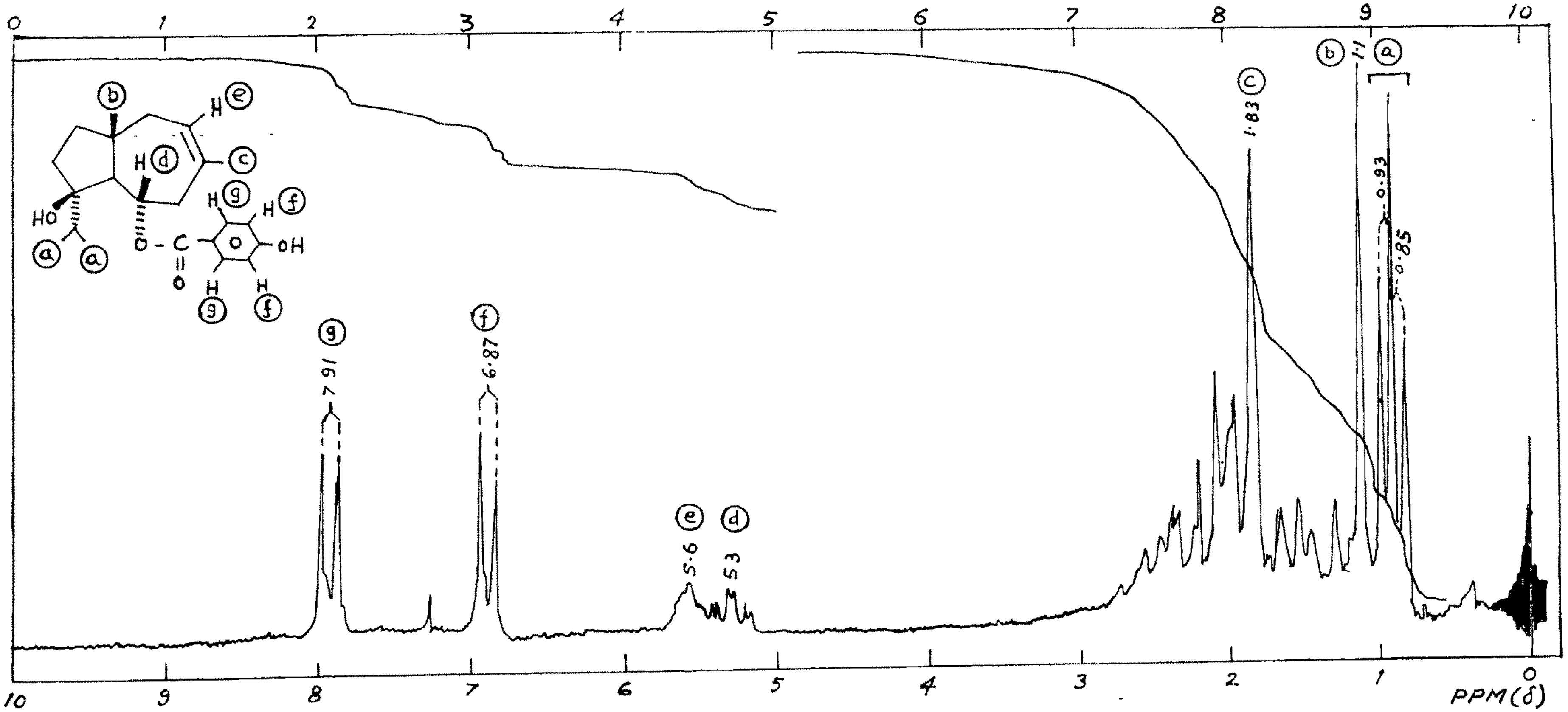
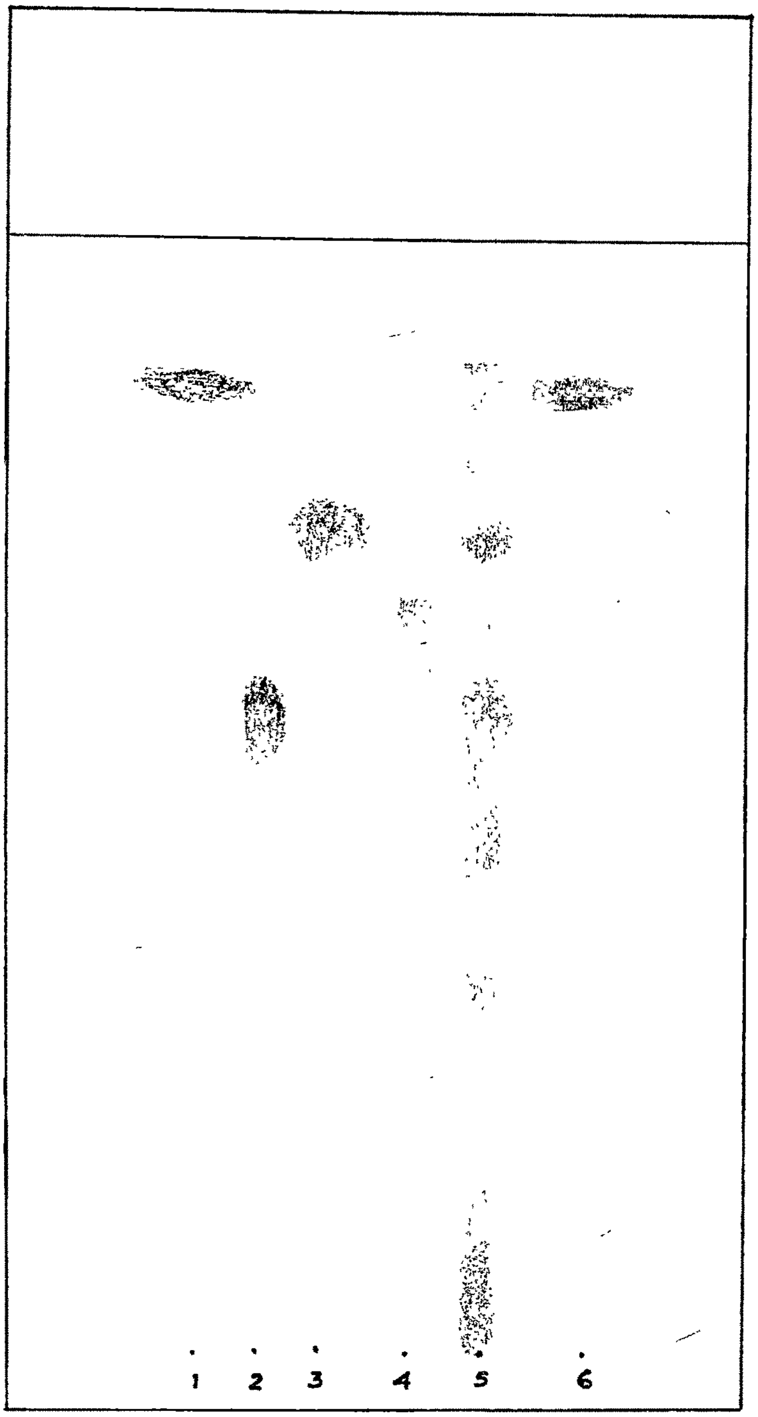


FIG. XIII. PMR SPECTRUM OF ESTER C



(SILICA GEL G PLATE)

SOLVENT SYSTEM: CHLOROFORM(95%)-ACETONE(5%)-METHANOL(0.5%)
VISUALISATION: 1% VANILIN IN 50% H_3PO_4 AQ. SPRAY
(1) DYE (SUDAN YELLOW) (2) ESTER C (3) ESTER B
(4) ESTER A (5) TOTAL EXTRACT (6) DYE (SUDAN (YELLOW)

FIG. XIV. THIN LAYER CHROMATOGRAM OF THE CONSTITUENTS OF FERULA JAESCHKEANA
(FROM TOTAL ACETONE EXTRACT)

EXPERIMENTAL

Isolation of esters A,B and C from the roots of *Ferula jaeshkeana* Vatke

The acetone extracts (105 g) was dissolved in ether (1 litre) and extracted with saturated $NaHCO_3$ aq (75ml x 7) to remove free acids (3.8 g). The neutral material obtained (101.2 g) was found to be a complex mixture on TLC (solvent : $CHCl_3$-$(CH_3)_2CO$-MeOH, 90:10:1). This material (48 g) was chromatographed over SiO_2-gel/IIB (120 cm x 5.5 cm) with TLC monitoring.

CHROMATOGRAM I

Fr.1	Light pet	3x600 ml	0.2 g	Hydrocarbons
Fr.2	Light pet-50% $CHCl_3$	3x600 ml		
Fr.3	$CHCl_3$	5x500 ml	1.942 g	Mixture
Fr.4	$CHCl_3$-5% acetone	5x500 ml		
Fr.5	-do-	4x500 ml	9.605 g	Mixture,rich in ester A and B.
Fr.6	-do-	2x500 ml	3.72 g	Mixture
Fr.7	-do-	10x500 ml	9.081 g	Mixture rich in ester C
Fr.8	-do-	3x500 ml	1.478 g	Mixture
Fr.9	$CHCl_3$-10% acetone	1x500 ml		
Fr.10	-do-	6x500 ml	1.477 g	Mixture
Fr.11	$CHCl_3$-15% acetone	12x500 ml	7.45 g	Polar compounds.

Frac.5(9.0g) from the above chromatography was rechromatographed over silica gel (II B, 270 g, 75 cm x 3 cm)

CHROMATOGRAM II

Fr. 1	Light pet	3x250 ml	0.172 g	Mixture
Fr.2	Light pet-25% $CHCl_3$	4x250 ml		
Fr. 3	Light pet-50% $CHCl_3$	3x250 ml	0.396 g	Mixture
Fr. 4	Light pet-50% $CHCl_3$	8x250 ml	3.443 g	Mixture
Fr. 5	Light pet-75% $CHCl_3$	4x250 ml		
Fr. 6	$CHCl_3$	5x250 ml		
Fr. 7	$CHCl_3$-2% acetone	3x250 ml		
Fr. 8	-do-	2x250 ml	2.079 g	Mixture
Fr. 9	-do-	4x250 ml	1.383 g	Mixture rich in B
Fr.10	-do-	2x250 ml	0.554 g	Single
Fr.11	Methanol	-	0.5 g	Rejected

Frac.10 was crystallised from acetonitrile to give ester A, m.p. 84-85° $[\alpha]_D$-1.98°(c, 1.51%). (Found:C,70.64; H,7.52. $C_{27}H_{36}O_6$ requires:C; 71.04; H,7.89%).Mass:m/e 438($M-18^+$,1%) 413 ($M-43^+$, 16%),218(27%), 176(78%), 175(47%),157(8%),147(22%), 133(13%),121(100%),105(10%),83(61%), 55(30%), 43(18%).

Frac.9(1 g, chromatogram II) was rechromatographed on SiO_2-gel (55 g, 1.5 cm x 64 cm).

CHROMATOGRAM III

Fr.1	Light pet	2x125 ml	Nil	-
Fr.2	Light pet-50% $CHCl_3$	6x100 ml	-do-	-
Fr.3	Light pet-75% $CHCl_3$	2x100 ml	-do-	-
Fr.4	$CHCl_3$	4x100 ml	-do-	-
Fr.5	$CHCl_3$-1% acetone	6x100 ml	0.516 g	Single
Fr.6	Acetone	-	0.45 g	Polar compounds.

Frac.5 was obtained in the foamy state. Despite several attempts, it could not be crystallised. It was distilled, b.p. 210-220°(bath)/o.2 mm to give ester B, $[\alpha]_D$+40.12° (c, 1.62%)(Found:C,71.0; H,8.46. $C_{23}H_{32}O_5$ requires: C,71.14; H,8.24%). Mass: m/e 345 ($M-43^+$,11%) 220(11%), 178(9.%), 177(41%), 168(22%), 159(28%),151(100%),134(24%), 121(17%), 105(11%),55(15%), 43(33%).

Frac.7 (2 g) from chromatogram I was rechromatographed on SiO_2-gel/IIB (100 g, 1.4 cm x 35 cm).

CHROMATOGRAM IV

Fr. 1	Light pet-50% $CHCl_3$	4x125 ml	Nil	-
Fr. 2	$CHCl_3$	2x125 ml	-do-	-
Fr. 3	$CHCl_3$-2% acetone	6x100 ml	0.065 g	Mixture
Fr. 4	$CHCl_3$-4% acetone	15x100 ml	1.518 g	Single
Fr. 5	Acetone	-	0.25 g	Rejected.

Frac.4 was also obtained in the foamy state and all the attempts to crystallise it failed. It was distilled b.p.215°-225° (bath)/0.1 mm to give ester C, $[\alpha]_D$+37.76° (c,1.43%)(Found:C, 73.24; H,7.83. $C_{22}H_{30}O_4$ requires: C,73.74; H, 8.37%). Mass:m/e 340(M-18$^+$, 1%), 315(12%), 220(5%), 177(59%), 159(47%), 149(7%), 134(29%), 121(100%), 55(9%), 43(22%).

Partial hydrolysis of ester A

To a solution of KOH (0.272 g) in dioxane (2 ml) containing water (2 ml) was added a soln of ester A (0.054 g) in dioxane (4 ml). The reaction mixture was initially stirred at room temperature for 1.5 hr, but no change was observed as only the starting material was shown on TLC (15% acetone in C_6H_6). It was then heated (80-90°) for 15 hr, cooled and diluted with water (10 ml). The aqueous soln was extracted with ether (3 x 10 ml) and the ether soln washed with water(3x5 ml), brine and dried. Solvent removal gave an oily residue (0.038 g), which was found to be a mixture of atleast three compounds as shown by TLC.

The aqueous layer was acidified using 50% H_3PO_4 aq. extracted with ether (3x5 ml), the ether soln was washed with water (3x5ml), brine and dried. Solvent evaporation furnished acids (0.013g). The acids were converted into the corresponding methyl esters using diazomethane in ether. The PMR of the crude mixture indicated the presence of p-hydroxy methylbenzoate as major compound.

Ar-COO$\underline{\text{M}}$e (3H,s,3.9 ppm), Ar-O$\underline{\text{H}}$ (1H, bs, 5.35 ppm) and Ar$\underline{\text{H}}$(4H, a typical AA'BB' 'quartet' centered at 7.41 ppm).

The neutral compound obtained from the above hydrolysis was chromatographed on SiO_2-gel/IIB(0.8 cm x 15.5cm)

Fr.1	C_6H_6	5x5ml	Nil	-
Fr.2	C_6H_6-1% acetone	8x5ml	0.014g	Single Rf 0.47
Fr.3	C_6H_6-2% acetone	2x5ml		
Fr.4	C_6H_6-5% acetone	5x5ml	0.005g	Single
Fr.5	Acetone	5x5ml	0.006g	Single Rf 0.22

Frac. 2 and 3 were combined and distilled, b.p. 200-210°(bath)/0,1 mm. IR (CHCl$_3$):OH(3590, 3530,1040 cm^{-1}); C=O(1705,1230,1160 cm^{-1}), C=CH(1640 cm^{-1}); PMR:-C-$\underline{\text{M}}$e (3H,s,1.1 ppm),-CHMe$_2$(6H, 2d, at 0.96 and 1.0 ppm, J=7Hz each),-C=C$\underline{\text{M}}$e(3H, s, 1.84 ppm)-CO-C$\underline{\text{M}}$e=CH$\underline{\text{M}}$e(6H,m,between 1.92 and 2.05 ppm),-C$\underline{\text{H}}$OH(1H,m, 4.1 ppm),-C$\underline{\text{H}}$-O-CO-C(1H, 2d, centered at 4.53 ppm, $J_1=J_3$=3Hz and J_2=8 Hz)-C=C$\underline{\text{H}}$ (1H, m, 5.46 ppm) and COCMe=C$\underline{\text{H}}$Me(1H, q, centered at 6.1 ppm, J=7Hz). Mass:m/e 318(M-18$^+$,1.5%) 293(1.5%), 218(18%), 200(5%), 193(26%), 175(25%), 147(9%),132(15.5%),121(15.5%), 83(100%),55(77%), 43(50%)(Found:C,71.46; H,9.04. $C_{20}H_{32}O_4$ requires:C,71.44; H,9.52%)

Frac.4 was found to be starting ester A (PMR,TLC)

Frac.5 was established to be jaeshkeanatriol(m.p.,PMR).

Total hydrolysis of ester A

Ester A(0.1 g) was refluxed with 10% methanolic KOH (10ml) under N_2 atmosphere for 4 hr. Excess of solvent was evaporated, the residue diluted with water and extracted with ether. The ether soln was worked up in the usual manner for neutral (0.045 g) and acidic (0.032 g) portions. The neutral compound was found to be jaeshkeanatriol (m.p., PMR). The acids were converted into corresponding methyl esters (0.028 g) using methanol(10ml) and sulfuric acid(0.02ml). It was found to be a mixture of two compounds (GLC, Hewlet Packard 7624-A, column: 10% silicone SE 30 on Chromosorb W (60-80 mesh); temp 160°C,H_2:60ml/min). The major compound (RT 7.2, 82%) was found to be p-hydroxy methylbenzoate and the minor component(RT 0.4, 18%) was established to be methyltiglate by coinjection with authentic samples of p-hydroxy methylbenzoate and methyltiglate.

Hydrolysis of ester B

Ester B (0.1 g) was refluxed with methanolic KOH (0.612 g in 10 ml MeOH) for 4 hr under N_2 atmosphere. Excess of solvent was evaporated, residue diluted with water and extracted with ether. Usual work up afforded neutral(0.063 g) and acidic (0.031g) compounds. The neutral compound m.p. 91-92° was found to be jaeshkeanadiol (mixed m.p.,PMR).

The acidic compound, m.p. 205–206° was converted into its methyl ester (CH_2N_2/Et_2O) and was identified as p-hydroxy methyl vanillate. IR($CHCl_3$):OH 3530, 1030 cm^{-1}; C=O 1705, 1100 cm^{-1} and C=CH 1600 cm^{-1}. PMR:Ar-OMe(3 H,s, 3.9 ppm), Ar.COOMe(3H,s,3.93 ppm), ArOH(1H, bs, 5.41 ppm) and ArH (3H, m, between 6.91 and 7.71 ppm).

Hydrolysis of ester C

Ester C(0.106 g) was also hydrolysed with methanolic KOH to give neutral (0.072 g) and acidic (0.032 g) components. The neutral compound was established to be jaeshkeanadiol (m.p.,mixed m.p.,PMR) The acidic m.p. 210–211° was converted into methylester (CH_2N_2/Et_2O). It was identified as p-hydroxy methylbenzoate. IR($CHCl_3$):OH 3580, 3360 cm^{-1}; C=O 1695, 1115 cm^{-1} and -C=CH 1605 cm^{-1}. PMR: ArCOOMe (3H, s, 3.9 ppm), ArOH (1H, s, 5.66 ppm), ArH(4H, AA'BB' 'quartet' centered at 7.36 ppm).

SUMMARY

From the total acetone extracts of the roots of <u>Ferula jaeschkeana</u>, three esters, which have been designated as esters A, B and C, have been isolated. These esters are shown to possess structures (2), (3) and (4) respectively on the basis of their spectral data and their saponification results.

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