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Extraction, Isolation and Characterization of Valuable Worked on Acacia Tortilis

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Abstract

Acacia tortilis is one of the important species of genus *Acacia* belonging to family Leguminaceae. Though there is no more study performed on this plant but it plays important role in the countries where it found. These countries include North Africa, Arabian Peninsula and Asian countries. The various part of *Acacia tortilis* plant say leaves, pods, gum exudates and bark were used as antidiabetic, antidiarrhoeal, antiasthmatic and also had several other medicinal benefits. The present discussion deals with the isolation and characterization of the following compounds from the leaves of *Acacia tortilis*. Lupan-3-ol, 12,20-diene, Lupan-12, 20-dien 3-one, Friedelin, β-amyrin, β-sitosterol, Apigenin, Luteolin, Quercetin, 5,7-dihydroxy-4-p-methyl benzylisoflavone, Vitexin, 2',6'-dihydroxy chalcone-4'-O-glucoside.

KEYWORDS: Acacia tortilis, Leaves, Terpenoids, Flavonoids, Flavonoid glycosides

INTRODUCTION

The genus Acacia comprising over 1200 species, found in the warmer drier parts of the World, chiefly in Arabia countries, Australia, Peninsula and Africa [1]. In India, there are about 22 indigenous species, distributed throughout the plains. Some of **Acacia** are of considerable value for reafforestation and reclamation of wasteland. They are the good source for tannin, gum and timber [2]. ***Acacia tortilis*** wild. **(Syn: A. Raddiana Savi)** was found to be a very useful source of protein. The acid digest of cell wall constituents fibers and cellulose found in the leaves provide nutrients for the animals as fodders. It is also used for the relaxation of smooth muscle [3]. Traditionally Acacia is used as anthelmintic, antidiarrhoeal, anti asthmaticand in pulmonary diseases [4]. The generic name 'Acacia' derived from the Greek word 'akis', meaning a point or a barb. The name 'tortilis' means twisted and refers to the pod structure. It is also known as umbrella thorn (Africa); haaken- steekdoring (South Africa); Israeli babool (India), samor (Egypt and Sudan); acacia de copa plana, espino de parasol (Spanish); acacia faux gommier (French); acacia ad ombrello (Italian); qurac (Somali); Mgunga Mwavuli (Swahili); שיטת הסוכך (Hebrew); سمر [Persian], and in Arabic it is commonly known as sunut, samar, sammar, samra, sayyal, seyal, seyyal [5].

MATERIAL AND METHODS

The dried and powdered leaves of ***Acacia tortilis*** (3 kg) procured from Yemen, were exhaustively extracted with light petroleum ether (60-80), benzene and finally with methanol. The petrol and benzene concentrate gave positive test for triterpenes [6]. On TLC examination, these concentrates showed number of spots in different solvent systems (Petrol-benzene and petrol-ether) with the same R_f values. The above two concentrates were therefore mixed together. The combined concentrate was chromatographed over silica-gel column, using successively petrol (A), petrol-benzene (9:1-B_1, 8:2-B_2, 7:3-B_3, 6:4-B_4, 1:1-B_5) and benzene (C) as eluting solvents. Appropriate fractions (**ir**. Spectra and TLC) were combined. The fractions A and B_1 on concentration gave a yellowish green oil of fatty nature and was not further examined. The fractions B_2 and B_3 on TLC examination (silica-gel, petrol-benzene 1:1) showed two major spots with the same R_f values. The above two fractions were therefore mixed together and subjected to column chromatography over silica-gel followed by fractional crystallization, afforded two crystalline TLC homogenous substances, marked as **(1)** and **(2)**. The

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fractions B_4, B_5 and C were found to be having the same composition with varying concentrations of the compounds. The three fractions were combined together. Repeated column chromatography over silica gel column using petrol-benzene mixtures in different ratios gave three compounds containing very minor impurities. Several crystallizations by appropriate solvent, gave pure compounds labeled as **(3), (4)** and **(5)**.

The brown gummy mass obtained after evaporation of the methanol extract gave positive colour test for Flavonoids [7]. TLC examination in toluene-ethylformate-formic acid (TEF 5:4:1) and benzene-pyridine-formic acid (BPF 36:9:5) systems showed it to be a mixture of several compounds. After several purification steps (refluxing it with petroleum ether, benzene and chloroform) including silica gel column chromatography. Fractional elution with benzene-ethylacetate (1:1) and ethylacetate yielded four compounds. They were purified by repeated crystallization and labeled as **(6), (7), (8)** and **(9)**. Further elution of the column with ethylacetate-methanol mixture gave two compounds labeled as **(10**) and **(11)**.

RESULT AND DISCUSSION

Compound (1):

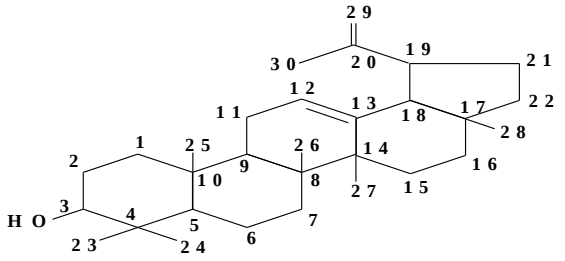
The compound **(1)** was crystallized from benzene-petrol as shining white needles m.p. 165-66^{0}C, $[\alpha]^{20}_D$ + 24.54 ($CHCl_3$). It gave a positive Leibermann-Burchard [8] and Nollers tests [9] and yellow colour with tetranitromethane, indicating the presence of double bond. Elemental analysis agreed with the formula, $C_{30}H_{48}O$. The **infrared** spectrum of the compound **(1)** showed absorptions at υ^{kBr}_{max} 3360 and 1030 cm^{-1} (OH), 1630, 1445 cm^{-1} (C=C) and 1375 cm^{-1} (geminal dimethyl), 875 cm^{-1} (terminal methylene).

The **^{1}H-NMR** spectrum of the compound **(1)** revealed seven methyl groups at δ 0.82 (3H), 0.91 (6H), 0.93 (3H), 0.99 (6H) and 1.68 (3H). The CH_2 protons were evident by the signals in the range of δ 1.34-1.65. The terminal double bond (>C=CH_2) was indicated by the signals at δ 4.57 and 4.68 while the signal at δ 4.85 corresponded to characteristic olefinic proton. A multiplet at δ 3.20 was ascribed to C-3-OH. It was also supported by the **mass** spectrum **(Scheme-I)** which showed the molecular ion peak ($M^{+\bullet}$) at m/z 424 (100%), with principal ions at m/z 409 ($M^{+\bullet}$-CH_3, 30%), 256 (9%), 207 (15%) and at m/z 188 (78%). The fragment ions at m/z 207,217 and 256 representing the presence of double bond at Δ^{12}-position.

The assigned structure was further confirmed by the **^{13}C-NMR** spectrum in which the C-3-OH appeared at δ 78.88 and the olefinic carbons at 129.63, 142.68, 150.90, 109.25. The assignment of other carbons are shown in (**Table-1**) [10].

Acetylation of **(1)** with acetic anhydride and pyridine gave a monoacetate m.p 152^{0}C, its **^{1}H-NMR** spectrum showed five independent singlets of methyl groups at δ 0.87 (3H), 0.93 (6H), 0.96 (3H), 1.02 (6H) and 1.68 (3H). The CH_2- protons appeared in the range of δ 1.36-1.59 and the terminal olefinic protons appeared at δ 4.56 and 4.68, while the signal at δ 4.85 indicated the Δ^{12} proton. A singlet at δ 2.17 corresponded to the acetoxyl group, the remaining multiplet at δ 3.17-3.21 ascribe to CHOAc proton.

On the basis of above result **(1)** was characterized as a new triterpene named as **Lupan-3-ol,12,20-diene** [3] **(I)**



(I)

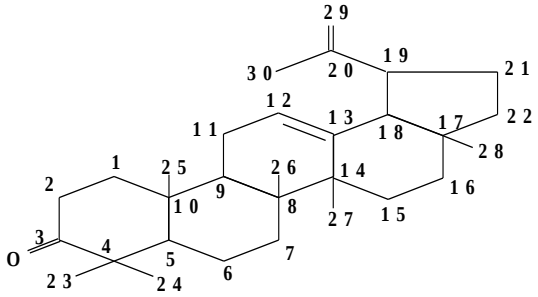
Compound (2):

The compound **(2)** obtained from the column with petrol-benzene (7:3), was crystallized with chloroform-methanol and afforded white shining crystals (50 mg) m.p.195^{0}C, analyzed for $C_{30}H_{46}O$. It gave positive Liebermann-Burchard [8] and Nollers [9] test indicating the presence of a triterpene. A yellow colour with tetranitromethane showed the presence of double bond. It gave a positive test to Zimmermann reaction [11] (a violet colour with m-dinitrobenzene in caustic potash) indicating the presence of keto group at 3-position which was further confirmed by its **ir** spectrum, which showed the characteristic bands at 1700 cm^{-1} (C=O), 1450 cm^{-1} (C=C), 1375 cm^{-1} (geminal dimethyl) and 895 cm^{-1} (terminal methylene).

The **^{1}H-NMR** spectrum of the compound **(2)** showed 7-methyl groups at δ 0.79 (3H), 0.94 (6H), 1.02 (3H), 1.03 (3H), 1.05 (3H) and 1.68 (3H). The CH_2 protons appeared in the range of δ 1.3-1.57. The double bond at Δ^{12} position was exhibited by the singlet at δ 4.86 and terminal methylene group was centered at δ 4.52 and 4.57. The **^{13}C-NMR** spectrum exhibited the presence of thirty carbons, the carbonyl group appeared at 206.1, assignment of other carbons is given in **(Table-2)**.

The **mass** spectrum of **(2)** showed the molecular ion peak at m/z 422, other fragments are rationalized by the **(Scheme-II)**.

On the basis of the above discussion the compound **(2)** was characterized as a novel triterpene **Lup-12,20-dien 3-one** [3] **(II).**



(I I)

The above assigned structure (II) was further confirmed by the bromination of Lupan-3-ol, 12,20-diene (I) followed by Jon's oxidation and debromination. The final product so obtained was found to be completely similar with that of **Lup-12,20-dien 3-one** (m.p., m.m.p, R_f value and co-TLC)**.**

(Table-1)^{13}C-NMR spectral data of (1)

Assignment	Signals	Assignment	Signals
C-1	35.52	C-16	33.27
C-2	25.09	C-17	48.25
C-3	78.88	C-18	51.15
C-4	37.31	C-19	55.44
C-5	55.25	C-20	150.90
C-6	18.26	C-21	27.37
C-7	34.23	C-22	38.35
C-8	39.94	C-23	31.27
C-9	50.38	C-24	19.25
C-10	37.64	C-25	16.63
C-11	20.87	C-26	16.05
C-12	129.63	C-27	15.34
C-13	142.68	C-28	17.94
C-14	47.92	C-29	109.25
C-15	29.76	C-30	21.04

Spectrum run at 300 MHz in $CDCl_3$

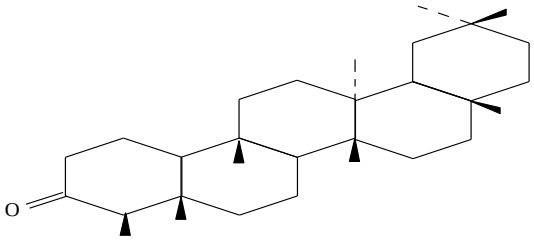
(Table-2)^{13}C-NMR spectral data of (2)

Assignment	Signals	Assignment	Signals
C-1	38.52	C-16	33.32
C-2	25.27	C-17	43.35
C-3	206.1	C-18	46.80
C-4	37.62	C-19	40.64
C-5	54.88	C-20	123.45
C-6	19.68	C-21	27.48
C-7	33.81	C-22	39.30
C-8	39.85	C-23	31.30
C-9	50.50	C-24	21.68
C-10	37.36	C-25	15.93
C-11	20.92	C-26	16.54
C-12	129.86	C-27	14.46
C-13	142.55	C-28	23.66
C-14	47.25	C-29	121.51
C-15	29.18	C-30	26.21

Spectrum run at 300 MHz in $CDCl_3$

Compound (3):

Elution of the column with petrol-benzene (1:1) followed by crystallization from chloroform-methanol gave white needle shaped crystals (150 mg), m.p. 262-64^{0}C. $[\alpha]^{23}_{546}$–29.4^0 ($CDCl_3$). It analysed for $C_{30}H_{50}O$ ($M^{+\bullet}$, m/z 426), colour tests [8,9] indicated it to be a triterpene. The melting point agreed with that of friedelin. Its identity as **friedelin (III)** was established by comparison of its **^{1}H-NMR (Table-3), ir** and **mass** spectra **(**with an authentic sample [12-14] of friedelin.



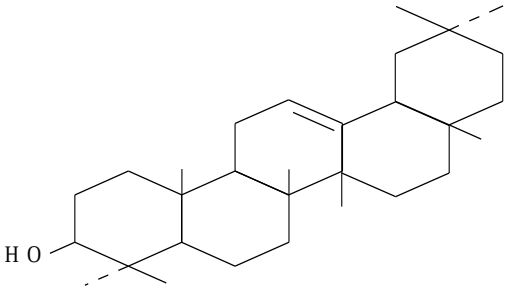
(III)

Compound (4):

It was eluted from the column with Petrol-benzene (2:3) and crystallized with chloroform-methanol as white crystalline solid m.p. 198^{0}C. $[\alpha]^{19}_D$+88.4^0 ($CDCl_3$). It gave positive Liebermann-Burchard test [8]. The **Infrared** spectrum showed the bands at 3360 cm^{-1} (OH), 2960 cm^{-1}, 2880 cm^{-1}, 1650 cm^{-1}, 1465 cm^{-1} (C=C), 1040 and 980 cm^{-1} indicating the presence of (OH) and (olefinic group).

The **mass** spectrum of **(4)** gave molecular ion peak at m/z 426 and analysed for $C_{30}H_{50}O$. Its **^{1}H-NMR** data are given in **(Table-4)**.

From the above data and their direct comparison with an authentic sample, **(4**) was identified as **β-Amyrin** [15] **(IV)**.



(IV)

Table-3 ^{1}H-NMR spectral data of (3)

Assignment	No. of Protons	Signals
CH_3	(3H, s)	0.72
CH_3	(3H, s)	0.87
CH_3	(3H, s)	0.89
CH_3	(3H, s)	0.92
2 x CH_3	(6H, s)	0.95
CH_3	(3H, s)	1.05
CH_3	(3H, s)	1.18
-CH_2 proton	22 protons	1.25, 1.34,1.45,1.52,1.58
C_2-2H C_4-IH	(3H,m)	2.26-2.41 (m)

S=singlet, m=multiplet, spectrum run in $CDCl_3$ at 200 MHz using TMS as internal standard (δ-scale).

Table-4^{1}H-NMR spectral data of (4)

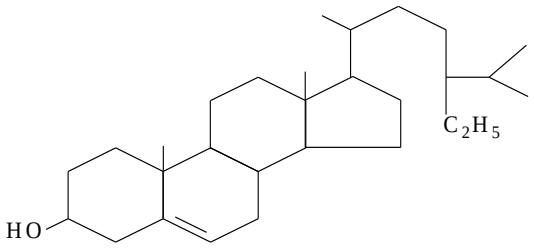
Assignment	No. of Protons	Chemical shift of Protons
8 x CH_3	24	0.78 (s, 3H), 0.83 (s, 3H), 0.88 (s, 6H), 0.95 (s, 3H), 0.98 (s, 3H), 1.0 (s, 3H), 1.14 (s, 3H)
-CH_2 and –CH Protons of cyclic system and side chain		1.08, 2.01, 3.01 (dd, J=9 Hz & 7 Hz)
-OH	1	4.88 (s, br)
Olefinic proton	1	5.21 (m)

s= singlet, dd=double doublet, br=broad, m=multiplet, spectrum run in $CDCl_3$ at 100 MHz using TMS as internal standard (δ-scale).

Compound (5): The fraction eluted from the column with Petrol-benzene (3:7) mixture gave white crystalline solid m.p. 136-37^{0}C, $[\alpha]_D$ –32.1^0 ($CDCl_3$). compound **(5**) gave an acetate with acetic anhydride and pyridine m.p. 114-16^{0}C, $[\alpha]_D$ –48.5^0 ($CHCl_3$) and benzoate m.p. 145-46^{0}C. It gave positive Liebermann-

Burchard test [8] and responded to the tetranitromethane colour test. The **ir** spectrum showed the presence of gem-dimethyl groups, hydroxyl group and olefinic double bond and showed bands at 3340 cm^{-1} (OH), 1055 cm^{-1}, 840 cm^{-1}, 1460 cm^{-1} (C=C), 1375 cm^{-1} (C-Me_2). The **^{1}H-NMR** data are given in **(Table-5)**. The **mass** spectrum showed the molecular ion peak at m/z 414.

From the above data and direct comparison with an authentic sample [15], compound **(5)** was characterized as **β-sitosterol (V)**.



(V)

Table-5 ^{1}H-NMR spectral data of (5)

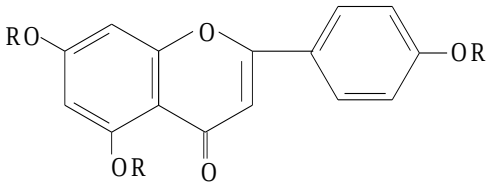
Assignment	Chemical shift of Protons
18-CH_3	0.70 (s, 3H)
28-CH_3	0.80 (d, J=6.8 Hz, 3H)
26, 27-CH_3	0.88 (d, J=6.5 Hz, 6H)
21-CH_3	0.92 (d, J=6.5 Hz, 3H)
19-CH_3	1.02 (s, 3H)
3-ax-H	3.56(m, 1H)
Olefinic proton	5.36 (m, 1H)
-CH_2 and –CH proton of cyclic system and side chain	1.07-2.34

s= singlet, d= doublet, m= multiplet, spectrum run at 90 MHz using TMS as internal standard (δ-scale).

Compound (6):

It was obtained by elution of column of methanol extract by benzene-ethylacetate (1:1) mixture and crystallized with benzene-acetone as yellow crystals, m.p. 352^{0}C. It gave greenish brown colour with alcoholic $FeCl_3$ and pink colour with zinc and hydrochloric acid, pointing out the presence of flavone nucleus. It was characterized as **apigenin (VI-a)** by comparison with an authentic sample (R_f-value, m.p., m.m.p, co-chromatography), further confirmed by **^{1}H-NMR** spectrum of its acetate (VI-b) **(Table-6)** m.p. 183-84^{0}C.

On the basis of above data, the compound **(6)** was characterized as **5,7,4'-trihydroxy flavone (Apigenin)** [16,17] **(VI-a)** which was further supported by **mass** spectrum which gave a molecular ion peak at m/z 270.



(VI)

(a) R=H
(b) R=Ac

Table-6^{1}H-NMR spectral data of (10Ac)

Assignment	No. of Protons	Signals
H-2',6'	2	7.85 (d, J=9 Hz)
H-3',5'	2	7.04 (d, J=9 Hz)
H-3	1	6.60 (s)
H-8	1	6.66 (d, J=2.5 Hz)
H-6	1	6.51(d, J=2.5 Hz)
OAc-5	3	2.42 (s)
OAc-4',7	6	2.35 (s)

s= singlet, d= doublet, spectrum run in $CDCl_3$ at100 MHz using TMS as internal standard (δ-scale).

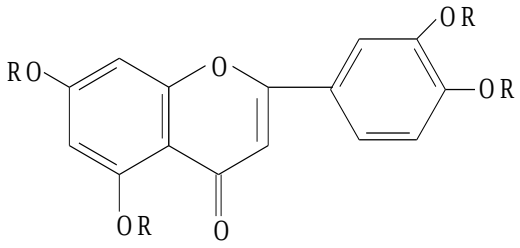
Compound (7):

It was eluted from the column by benzene-ethyacetate (1:1) mixture as yellow solid which was purified by crystallization from ethylacetate-acetone m.p. >315^{0}C. Elemental analysis agreed to the molecular formula $C_{15}H_{10}O_6$. It responded positively to Shinoda's test [6] and gave greenish brown colour with $FeCl_3$. The **uv** spectrum showed λ^{MeOH}_{max} at 258, 265 and 346 nm. Analysis of functional groups revealed the presence of phenolic OH (3400 cm^{-1}), α,

β-unsaturated ketonic group >C=O (1640 cm^{-1}) and aromatic nucleus (800 and 840 cm^{-1}). The **uv** spectrum in the presence of diagnostic shift reagents [18] indicated the presence of free hydroxyl groups at 5 and 7 positions and 3',4'-dihydroxyl groups.

Acetylation of **(7)** gave a tetraacetate (7Ac) (VII-b) m.p. ≈ 200-201^{0}C. The **^{1}H-NMR** spectrum of 7Ac **(Table-7)** evidenced the presence of four aromatic acetoxyls integrating for 12 protons at δ 2.43 (3H), 2.35 (3H) and 2.33 (6H) assigned to OAc-5, OAc-7 and OAc-3',4' respectively. **^{1}H-NMR** also indicated a disubstituted ring-B as it showed a typical one proton double doublet at δ 7.75 (J_1=9 Hz and J_2=2.20 Hz, H-6') and a doublet of one proton δ 7.80 (J=2.20 Hz, H-2'). This could be attributed to 3',4'-substitution of ring-B. Another ortho coupled doublet integrating for one proton at δ 7.25 (J=9 Hz) was ascribed to H-5'. A pair of meta-coupled doublets centered at δ 6.45 (J=2.5 Hz) and 6.95 (J=2.5 Hz) were assigned to C-6 and C-8 protons of ring-A, while C-3 proton of pyrone ring resonated as a sharp singlet at δ 6.59. The **mass** spectrum showed a molecular ion peak at m/z 286 corresponding to the structure (VII-a). The fragment ions at m/z 134 fully supported a ring-B with two hydroxyl groups. Fragment at m/z 153 was consistent with the ring-A having two hydroxyl groups.

On the basis of these results compound **(7)** was characterized as **5,7,3',4' tetrahydroxy flavone (Luteolin)** [16] **(VII-a)**.



(V III)

(a) R = H
(b) R = A c

Table-7 ^{1}H-NMR spectral data of (7Ac)

Assignment	No. of Protons	Signals
H-6	1	6.45 (d, J=2.5 Hz)
H-8	1	6.95 (d, J=2.5 Hz)
H-3	1	6.59 (s)
H-5'	1	7.25 (d, J=9 Hz)
H-2'	1	7.80 (d, J=2.20 Hz)
H-6'	1	7.75 (dd, J_1=9 Hz & J_2=2.20 Hz)
OAc-5	3	2.43 (s)
OAc-7	3	2.35 (s)
OAc-3',4'	6	2.33 (s)

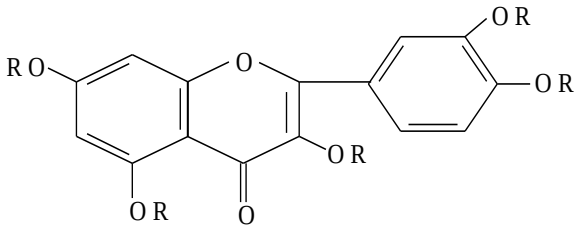
s= singlet, d= doublet, dd= double doublet, spectrum run in $CDCl_3$ at 270 MHz using TMS as internal standard (δ-scale).

Compound (8):

It was crystallized with methanol, as yellow crystals, m.p. 311-12^{0}C and was characterized as **quercetin** by co-chromatography and mixed melting point with an authentic sample. On acetylation with acetic anhydride and pyridine, it gave an acetate m.p. 194-95^{0}C. The identity of the compound as quercetin was further confirmed by spectral studies (**uv** and **^{1}H-NMR** spectra) **(Table-8 and Table-9)**.

The **^{1}H-NMR** spectrum of its acetate (VIII-b) showed the signals due to five acetoxyls at δ 2.35-2.40. A pair of meta-coupled doublet at δ 6.87 (J=2.5 Hz) and δ 6.65 (J=2.5 Hz) was assigned to H-8 and H-6 protons of ring-A respectively. The ring-B protons showed an ABX pattern, two doublet at δ 7.74 (J=2.5 Hz) for H-2' and δ 6.92 (J=8.5 Hz) for H-5' and a quartet at δ 7.63 (J=2.5 Hz and 8.5 Hz) for H-6'.

In the light of above results compound **(8)** was assigned the structure as **3, 5,7, 3',4'-pentahydroxy flavone (quercetin)** [19] **(VIII-a).**



(V III)

(a) R = H
(b) R = A c

Compound (9):

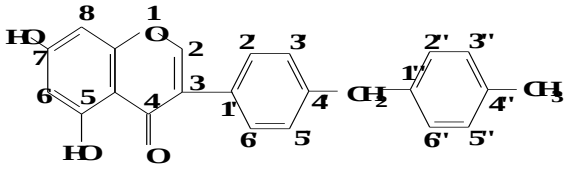
It was eluted from the column by ethylacetate and was crystallized with methanol as pale-yellow granular crystals m.p168^{0}C analysed for $C_{23}H_{18}O_4$. It gave a greenish brown colour with alcoholic ferric chloride, and a pink colour with sodium amalgum / HCl and yellow colour with conc. H_2SO_4. The colour

test and **uv** absorption, λ_{max}262 and inflection at 339 nm indicated isoflavone nucleus, further supported by a singlet in its **^{1}H-NMR** spectrum at δ 7.86 ascribed to H-2 proton of isoflavone. A red shift of 10 nm with $AlCl_3$ and 11 nm with NaOAc showed the presence of hydroxyl group at 5 and 7 positions which was further confirmed by the appearance of the singlets at δ 12.46 and δ 9.27 in the **^{1}H-NMR** spectrum **(Table-10)**.

The **^{1}H-NMR** spectrum displayed a singlet integrating for 3 protons at δ 2.50 corresponding to methyl group and a pair of meta-coupled doublets of one proton each at δ 6.17 (J=2.5 Hz) and 6.40 (J=2.5 Hz), attributed to H-6 and H-8 protons of ring-A respectively. Another pair of ortho coupled doublets of four protons each at δ 6.89 (J=9 Hz) and 7.56 (J=9 Hz) were assigned to H-3',5',3",5" and H-2', 6',2'',6" respectively. A solitory one proton singlet at δ 7.86 was ascribed to H-2 proton of isoflavone. The CH_2 protons appeared at δ 2.59.

The assigned structure was further supported by the **mass** spectrum **(Scheme-III)** which showed the molecular ion peak at m/z 358. The RDA fragments appeared at m/z 152, 206 and the base peak was observed at m/z 91 corresponded to tropolium ion.

On the basis of above studies, it was characterized as the novel isoflavone named as **5,7-dihydroxy-4'-p-methyl benzyl isoflavone** [17] **(IX).**



(IX)

Table-8 UV data of (8) and quercetin

Reagent	compound **(8)**	**Quercetin**
MeOH	256,270 sh, 301 sh, 372	255, 269 sh, 301 sh, 370
NaOMe	247 sh. 321 (Dec)	247 sh, 321 (Dec)
$AlCl_3$	274, 304 sh, 334, 458	272, 304 sh, 333, 458
$AlCl_3$/HCl	264, 358, 427	265, 301 sh, 359, 428
NaOAc	257 sh, 274, 329, 390	257, 274, 390 (Dec)
NaOAc/H_3BO_3	264, 303 sh, 389	261, 301 sh, 388

Table-9 ^{1}H-NMR spectral data of (8Ac)

Assignment	**No. of Protons**	**Signals**
H-2'	1	7.74 (d, J=2.5 Hz)
H-6'	1	7.63 (q, J_1=2.5 Hz, J_2=8.5 Hz)
H-5'	1	6.92 (d, J=8.5 Hz)
H-8	1	6.87 (d, J=2.5 Hz)
H-6	1	6.65 (d, J=2.5 Hz)
5 x OAc	15	2.35 (m), 2.40 (s)

s= singlet, d= doublet, q=quartet, m=multiplet, spectrum run in $CDCl_3$ at 100 MHz using TMS as internal standard (δ-scale).

Compound (10):

Elution of the column by ethylacetate-methanol (9:1) followed by crystallization with methanol-chloroform, it gave yellow crystals m.p. 263-64^{0}C. The molecular ion peak at m/z 432 and the elemental analysis agreed with the molecular formula as $C_{21}H_{20}O_{10}$. A dark reddish colour with magnesium and hydrochloric acid and a red colour on treatment with sodium amalgam, followed by acidification suggested a flavone nucleus for the compound.

The compound **(10)** gave a positive Molish test [20] and a dark brown colour with ferric chloride. The analysis of functional groups revealed the presence of α,β-unsaturated >C=O (1650), phenolic OH (3420), and a complex aromatic substitution, besides a strong band at 2950 cm^{-1}. The uv spectrum showed λ^{MeOH}_{max} at 268 and 335 nm. The red shift of 10 nm with NaOAc in band-II, 11 nm with $AlCl_3$ and 38 nm with NaOMe in band I (without decrease in intensity) indicated the presence of 5,7 and 4' hydroxyls groups.

Prolonged heating (5 hours) of the glycoside with 0.4 M. HCl failed to hydrolyses the glycoside, suggestive of a C-glycosyl nature of the compound. This was further supported by two bands at 1010 and 1038 cm^{-1} in its **ir** spectrum [21]. The compound **(10)** was oxidised with $FeCl_3$, the sugar obtained was identified as glucose (by m.p., co-chromatography and **glc** of trimethylsilyl derivative). Pyranose structure of glucose was confirmed by periodate oxidation of methyl ether of the glucoside.

On boiling the glucoside **(10)** with hydroiodic acid in phenol, the sugar moiety was decomposed. The resulting product was identified as apigenin (10ag) by comparison of m.p. and spectral data **(uv, ir, 1H-NMR**

and **mass)** with those of an authentic sample [22]. The **uv** spectrum of the aglycone was found to be identical with that of glucoside. The acetate of the glucoside, prepared by heating it with acetic anhydride and pyridine gave white crystals (10Ac), m.p. 154-156°C analysed for $C_{33}H_{32}O_{16}$.

The **^{1}H-NMR** spectrum **(Table-11)** exhibited a pair of ortho-coupled doublets integrating for two protons each at δ 7.40 (J=9 Hz) and δ 8.10 (J=9 Hz) corresponded to H-3',5' and H-2',6'. Two independent singlets of one proton each at δ 6.8 and δ 6.91 were assigned to H-3 and H-6 respectively. The anomeric proton H-1" (glu) appeared as a doublet at δ 4.64 (J=10 Hz) showing trans diaxial relationship with H-2", while the sugar protons appeared in the range of δ 3.60-5.70. Three singlets at δ 2.32, 2.43 and 2.51 integrating for three protons each were attributed to three aromatic acetoxyls at 4,7 and 5 positions. The four aliphatic acetoxyls appeared as a multiplet at δ 1.72-2.02. The presence of the signals at δ 1.72 for 2"-OAc and 2.02 for 6"-OAc indicated the presence of sugar moiety at C-8 [23]. This was supported by the negative Gibb's test. The location of the sugar at C-8 was further confirmed by the mass fragmentation, as the characteristic fragments at m/z 312 (aglycone attached to CH_2-CHO) was observed [24].

The other fragments observed were at m/z 283 [aglycone attached with CH_2] and 354 [$M^{+\bullet}$-C-glucosyl + H^+]. The fragment ion at m/z 270 seemed to be formed by the loss of 2 x CH_2=C=O groups from m/z 354. The RDA fragments representing ring-A and ring-B were observed at m/z 194 $[A_1]^+$ and at m/z 118 $[B_1]^+$ respectively.

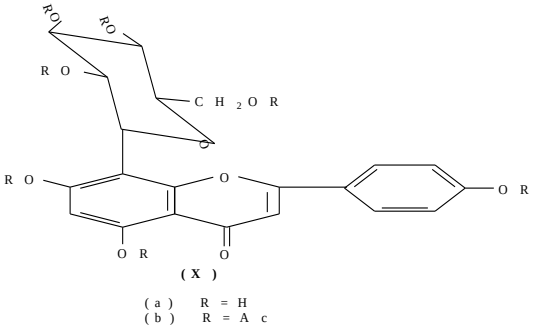


Table-10^{1}H-NMR spectral data of (9)

Assignment	No. of Protons	Signals
CH_3	(3H, s)	2.50
CH_2	(2H, s)	2.59
H-6	(1H, d, J=2.5 Hz)	6.17
H-8	(1H, d, J=2.5 Hz)	6.40
H-3',5',3",5"	(4H, d, J=9 Hz & 2.5 Hz)	6.89
H-2',6',2",6"	(4H, d, J=9 Hz & 2.5 Hz)	7.56
H-2	(1H, s)	7.86
CH_2	(2H, s)	2.59
7-OH	(1H, brs)	9.27
5-OH	(1H, s)	12.46

s= singlet, brs= broad singlet, d= doublet, spectrum run in DMSO-d_6 at 300 MHz using TMS as internal standard (δ-scale).

Table-11 ^{1}H-NMR spectral data of (10Ac)

Assignments	No. of Protons	Signals
H-3	1	6.8 (s)
H-6	1	6.91 (s)
H-3',5'	2	7.4 (d, J=9 Hz)
H-2',6'	2	8.1 (d, J=9 Hz)
H-1"	1	4.64 (d, J=10 Hz)
H-1",2",3",4",5",6"	7	3.65-5.70 (m)
Aromatic acetoxyls:		
OAc	3	2.32 (s)
OAc	3	2.43 (s)
OAc	3	2.51 (s)
Aliphatic acetoxyls	12	1.72-2.02 (m)

s = singlet, m = multiplet, d = doublet, spectrum run in $CDCl_3$ at 300 MHz., using TMS as internal standard (δ-scale).

On the basis of the above results, the compound **(10)** was characterized as **vitexin** [25] **(X)**.

Compound (11): Elution of the column with ethylacetate-methanol (8:2-7:3) followed by crystallization with methanol-chloroform afforded

pale yellow crystals (100 mg) m.p. >280^{0}C. The elemental analysis agreed with the molecular formula $C_{21}H_{22}O_9$. It gave red colour with conc. H_2SO_4 and orange to red colour with aqueous NaOH suggesting it to be a chalcone [26,27]. Positive Molish test [20] indicated it to be a chalcone glycoside. The **ir** spectrum showed the characteristic bands at 2965 (br, chelated OH), 1684 (C=O) and 1462 (C=C) cm^{-1}. **UV** spectrum showed the absorptions at 365 nm (Band-I) and at 245 nm (Band-II). A red shift of 71 nm with $AlCl_3$/HCl in Band-I showed the presence of chelated-hydroxyl group [28]. **Compound (11)** gave greenish brown colour with alcoholic $FeCl_3$, indicating the presence of 2' and 6' hydroxyl groups. The placement of hydroxyl groups at 2' and 6' positions was justified by its **^{1}H-NMR** spectrum **(Table-12)** which showed two exchangeable hydroxyl groups with D_2O by the off-field signals at δ 13.6 (2'-OH) and at δ 12.85 (6'-OH).

Acetylation of the glycoside **(XI-a)** with acetic anhydride and pyridine afforded an acetate **(XI-b)** m.p 178^{o}C. Its **^{1}H-NMR** spectrum indicated it to be an hexaacetate derivative as it exhibited the presence of six acetoxyl groups (two aromatic and four aliphatic). A six protons singlet at δ 2.52 was ascribed to the aromatic acetoxyl at 2' and 6'-position while the aliphatic acetoxyls integrating for twelve protons appeared as a multiplet in the range of δ 1.78-2.05. A pair of meta coupled doublets at δ 6.24 (J=2.0 Hz) and δ 6.26 (J=2.0 Hz) were assigned to 3' and 5' protons of ring-A respectively. The ring-B protons (2,3,4,5,6) appeared as multiplet in the range of δ 6.77-6.85. A pair of doublets at δ 6.97 (J=15 Hz) and 7.85 (J=15 Hz) were accounted for α and β-protons of chalcone. The sugar protons appeared as multiplet in the range of 3.5-4.32 and 5.22-5.50. While the anomeric proton of glucose (H-1") was centered as doublet at δ 4.1 (J=9 Hz). The coupling constant supporting the β-linkage of glucose.

Compound (11) on hydrolysis with 6% HCl yielded a sugar and an aglycone. The sugar was identified as glucose by R_f value and co-chromagraphy with an authentic sample of glucose.

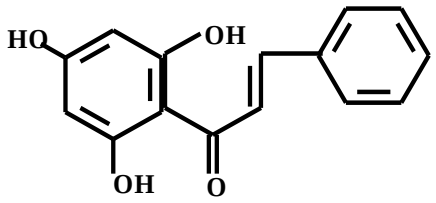
The ultra-violet and infrared spectra of the aglycone and its derivatives showed that it contained a conjugated carbonyl group and three phenolic hydroxyl groups. It gave greenish brown colour with ferric chloride. The hydroxyl groups were placed at 2' and 6' positions of the chalcone as discussed above. The remaining hydroxyl group was placed at 4'-position since it gave a red colour with vanillin-HCl reagent [29] and a red shift of 62 nm in band I with NaOMe in its **uv** spectrum (absent in glycoside). Thus, all the three hydroxyl groups were placed in ring-A with no substitution in ring-B (alkaline fusion of the aglycone gave benzoic acid). The presence of a free 4'-OH group in the aglycone which was not found in the glycoside indicated the sugar linkage in the chalcone at 4'-position.

The **^{1}H-NMR** of the aglycone exhibited a pair of meta-coupled doublets at δ 6.20 (J=2.0 Hz) and δ 6.23 (J=2.0 Hz) for H-3' and H-5' protons respectively. The ring-B protons (H-2',3',4',5',6') appeared in the range of δ 6.75-6.82. The remaining α,β protons of the chalcone resonated as a doublet at δ 6.95 (J=15 Hz, H-α) and δ 7.81 (J=15 Hz, H-β). Therefore, the aglycone was characterized as **2'**,**4'**,**6'-trihydroxychalcone** [30,31] **(XI)**.

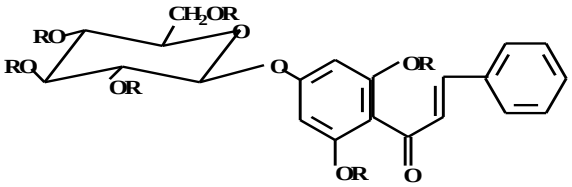
The quantitative estimation of the sugar by somogyi's copper-micro method [32] showed the presence of 1 mole of glucose per mole of the aglycone.

The **mass** spectrum **(Scheme-IV)** of the glycoside (XI-a) was in full agreement with the assigned structure of the glycoside. It showed the presence of glucose at m/z 180 and an aglycone at m/z 256. The retro-Diels-Alder fragmentation pattern was observed by peaks at m/z 152, 131,126 and 104, supporting the presence of three hydroxyl groups in ring-A and no-hydroxyl group in ring-B. It was further confirmed by the degredation [33] of the aglycone with 50% KOH which gave phloroglucinol and cinnamic acid which were identified by co-TLC with authentic samples.

On the basis of the above results the compound (**11**) was thus characterized as **2'**,**6'-dihydroxychalcone-4'-O-glucoside** [25] **(XI-a)**.



(XI)



(XI-a) R=H
(XI-b) R=Ac

Table-12 ^{1}H-NMR spectral data of (11Ac)

Assignment	No. of Protons	Signals
OAc-2',6'	6	2.52 (s)
H-3'	1	6.24 (d, J=2.0 Hz)
H-5'	1	6.26 (d, J=2.0 Hz)
H-2,3,4,5,6	5	6.77-6.85 (m)
H-α	1	6.97 (d, J=15 Hz)
H-β	1	7.85 (d, J=15 Hz)
Sugar protons:		
H-1" (anomeric)	1	4.1 (d, J=9 Hz)
H-1",2",3",4"	4	3.5-4.32 (m)
H-5",6"	3	5.22-5.50 (m)
Sugar acetoxyls:		
H-2",3",4",6"	12	1.78-2.05 (m)

s= singlet, d= doublet, m=multiplet, spectrum run in DMSO at 300 MHz using TMS as internal standard (δ-scale).

EXPERIMENTAL

The melting points were taken on a Kofler block and are uncorrected. All **UV** spectra were measured on Beckmann Model DU and Pye Unicam PU-8800 spectrophotometers in methanol / ethanol. **IR** spectra were taken on Shimadzu IR-408 Perkin Elmer 1800 (FTIR). The **MS** and **^{1}H-NMR** spectra were obtained from different institutes. The **MS** spectra were mostly measured in E.I. mode on Jeol D-300 while, the **^{1}H-NMR** spectra were usually recorded on JEOL 4H-100 MHz, Brucker dpx 200 MHz and 270 MHz and in $CDCl_3$ / DMSO-d_6 using TMS as internal standard. The silica gel used for different chromatographic purposes, was obtained from E. Merck (India), E. Merck (Germany) and SRL (India). TLC solvent systems used were benzene-pyridine-formic acid (BPF, 36:9:5) and toluene-ethylformate-formic acid (TEF, 5:4:1). Alcoholic ferric chloride solution, were used as spray reagents for visualization of spots on TLC.

Plant material

Leaves of ***A. tortilis*** were collected from Yemen in the month of March. It was identified by Prof. Wazahat Hussain, Department of Botany, A.M.U., Aligarh, India. The voucher specimen has been deposited at Department of Botany, A.M.U., Aligarh.

Extraction and isolation

The dried and powdered leaves of ***Acacia tortilis*** (3 kg) were exhaustively extracted with light petroleum ether (60-80), benzene and finally with methanol. The petrol and benzene concentrate gave positive test for triterpenes. On TLC examination, these concentrates showed number of spots in different solvent systems (Petrol-benzene and petrol-ether) with the same R_f values. The above two concentrates were therefore mixed together. The combined concentrate was chromatographed over silica-gel column, using successively petrol (A), petrol-benzene (9:1-B_1, 8:2-B_2, 7:3-B_3, 6:4-B_4, 1:1-B_5) and benzene (C) as eluting solvents. Appropriate fractions (**ir**. Spectra and TLC) were combined. The fractions A and B_1 on concentration gave a yellowish green oil of fatty nature and was not further examined. The fractions B_2 and B_3 on TLC examination (silica-gel, petrol-benzene 1:1) showed two major spots with the same R_f values. The above two fractions were therefore mixed together and subjected to column chromatography over silica-gel followed by fractional crystallization, afforded two crystalline TLC homogenous substances, marked as **(1)** and **(2)**. The fractions B_4, B_5 and C were found to be having the same composition with varying concentrations of the compounds. The three fractions were combined together. Repeated column chromatography over silica gel column using petrol-benzene mixtures in different ratios gave three compounds containing very minor impurities. Several crystallizations by appropriate solvent, gave pure compounds labeled as **(3), (4)** and **(5)**.

The methanol extract was concentrated by heating over a boiling water bath, a brown gummy mass was obtained. It gave positive colour test for flavonoids. The examination in toluene-ethylformate-formic acid (TEF 5:4:1) and benzene-pyridine-formic acid (BPF 36:9:5) systems showed it to be mixture of several compounds. The brown gummy mass was purified by refluxing it with petroleum ether, benzene and chloroform. The semi-solid mass left behind was subjected to silica gel column chromatography. Fractional elution with benzene-ethyl acetate (1:1) and ethylacetate yielded four compounds. They were purified by repeated crystallization and labelled as **(6)**, **(7), (8)** and **(9)**. Further elution of the column with ethylacetate-methanol mixture gave two compounds labeled as **(10**) and **(11)**.

Compound (1):

Elution of the column with petrol-benzene (4:1) afforded a solid substance which on crystallization with benzene-petrol gave white shining crystals **(1)** (500mg), m.p 165-66°C, $[\alpha]_D^{20}$ + 24.54

($CHCl_3$). It gave positive Lieberman-Burchard and Nollers tests and yellow colour with tetranitromethane.

Analysed for $C_{30}H_{48}O$: Calcd.: C, 84.9; H, 11.37%. Found: C, 84.7; H, 11.1%

IR, υ^{KBr}_{max} cm^{-1}:

3360 and 1030 cm^{-1} (OH), 1630 and 1445 (C=C), 1375 (geminal dimethyl), 875 (terminal methylene).

^{1}H-NMR (300 MHz, $CDCl_3$) on δ-scale:

0.82 (3H, s), 0.91 (6H, s), 0.93 (3H, s), 0.99 (6H, s), 1.68 (3H, s), 1.34- 1.65 (CH_2 – Protons), 4.57 and 4.68 (>C=CH_2), 4.85 (Δ^{12}-double bond), 3.20 (1H, m, CH-OH, 3-OH).

^{13}C-NMR (300 MHz, $CDCl_3$) on δ-scale:

35.52 (C-1), 25.09 (C-2), 78.88 (C-3), 37.31 (C-4), 55.25 (C-5), 18.26 (C-6), 34.23 (C-7), 39.94 (C-8), 50.38 (C-9), 37.64 (C-10), 20.87 (C-11), 129.63 (C-12), 142.68 (C-13), 47.92 (C-14), 29.76 (C-15), 33.27 (C-16), 48.25 (C-17), 51.15 (C-18), 55.44 (C-19), 150.90 (C-20), 27.37 (C-21), 38.35 (C-22), 31.27 (C-23), 19.25 (C-24), 16.63 (C-25), 16.05 (C-26), 15.34 (C-27), 17.94 (C-28), 109.25 (C-29), 21.04 (C-30).

Mass, m/z:

424 ($M^{+\bullet}$, 100%), 409 ($M^{+\bullet}$-CH_3, 30%), 217 (70.3%), 207 (15%), 205 (20%), 201 (42%), 256 (9%), 190 (29%), 189 (31%), 188 (78%), 174 (35%), 207 (15%).

Acetylation of Compound (1):

The compound **(1)** (60 mg) was treated with pyridine (2 ml) and acetic anhydride (4 ml), allowed to stand overnight at room temperature and then heated on a water bath for 2 hours. The reaction mixture was cooled and poured over crushed ice with constant stirring. Dirty white mass separated was filtered under suction and washed thoroughly with water. The solid thus obtained was crystallized from chloroform-methanol as fine needle shaped crystals (35 mg) m.p 152^0C.

^{1}H-NMR (300 MHz, $CDCl_3$) on δ-scale:

0.87 (3H, s), 0.93 (6H, s), 0.96 (3H, s), 1.02 (6H, s), 1.68 (3H, s), 1.36- 1.59 (m, CH_2 protons), 4.56 and 4.68 (>C=CH_2), 4.85 (s, Δ^{12}-double bond), 2.17 (3H, s, OAc), 3.17-3.21 (m, CH-OAc, C-3-OAc).

Compound (2):

Compound **(2)** was obtained by the elution of the column with petrol-benzene (7:3) and crystallized with chloroform-methanol as white shining crystals (50 mg), m.p.195°C. Elemental analysis agreed with the molecular formula $C_{30}H_{46}O$.

Analysed for $C_{30}H_{46}O$: Calcd.:C, 85.30; H, 10.9%. Found: C, 85.28; H, 10.8%

IR, υ^{KBr}_{max} cm^{-1}:

1700 (C=O), 1450 (C=C), 1375 (geminal dimethyl), 845 (terminal methylene, = CH_2)

^{1}H-NMR (300 MHz, $CDCl_3$) on δ-scale:

0.79 (3H, s), 0.94 (6H, s), 1.02 (3H, s), 1.03 (3H, s), 1.05 (3H, s), 1.68 (3H, s), 1.3-1.57 (CH_2 protons), 4.52, 4.57 (>C=CH_2), 4.86 (Δ^{12}-double bond), 1.96-2.26 (m, -CH_2).

^{13}C-NMR (300 MHz, $CDCl_3$) on δ-scale:

38.52 (C-1), 25.27 (C-2), 206.1 (C-3), 37.62 (C-4), 54.88 (C-5), 19.68 (C-6), 33.81 (C-7), 39.85 (C-8), 50.50 (C-9), 37.36 (C-10), 20.92 (C-11), 129.86 (C-12), 142.55 (C-13), 47.25 (C-14), 29.18 (C-15), 33.32 (C-16), 43.35 (C-17), 46.80 (C-18), 40.64 (C-19), 123.45 (C-20), 27.48 (C-21), 39.30 (C-22), 31.30 (C-23), 21.68 (C-24), 15.93 (C-25), 16.54 (C-26), 14.46 (C-27), 23.66 (C-28), 121.51 (C-29), 26.21 (C-30).

Mass, m/z:

422 ($M^{+\bullet}$, 44%), 407 ($M^{+\bullet}$-CH_3, 41%), 256 (10), 206 (7), 188 (79%), 174 (35%), 176 (100%)

Bromination of lupan-3-ol, 12, 20-diene:

Lupan-3-ol, 12, 20-diene (50 mg) was dissolved in ether (10 ml) and then bromine solution (5 ml) [prepared by dissolving sodium acetate (anhydrous) (1 gm) in acetic acid (glacial) (100 ml) and bromine (9.5 ml) is add] was added gradually with constant shaking under cold conditions 0-5°. When the addition was complete it was kept under the same conditions with occasional shaking for another 15 minutes and then cold water (≈50 ml) was added. Organic matter was extracted with ether washed with water, $NaHCO_3$ (5%) solution, and sodium thiosulphate solution (5%) and again with water successively. The ethereal layer was dried over sodium sulphate anhydrous and then the solvent was evaporated to give a solid mass (40 mg) which gave positive Beilstein test and a negative nitromethane test.

The solid thus obtained was suspended in acetone (20 ml) cooled in an ice bath and then Jones reagent (5 ml) was added dropwise with constant stirring. When the Jones reagent was added the stirring was continued for a further period of 10 minutes and cold water (50 ml) was added. The organic matter was extracted with ether and washed thoroughly with water to make it neutral. The ethereal layer was dried over anhydrous sodium sulphate and then the solvents were removed by evaporation to give a semisolid mass (25 mg) which gave a positive Beilstein test.

The semisolid thus obtained was dissolved in dry ether (20 ml) and acetic acid (5 ml) then zinc dust (2 gm) was gradually added over a period of 30 minutes with constant shaking. When the addition of zinc was complete it was filtered and the filtrate was washed thoroughly with water to remove acid, the ether layer was dried over anhydrous sodium sulphate and the residue was crystallized from methanol to give the compound **(2)** (10 mg) (along with mother liquour having other compounds), mp,. m.m.p. and TLC identical with the sample **(2)** obtained from natural sources.

Compound (3):

Compound **(3)** was obtained on elution of the column with petrol-benzene (1:1). After repeated

crystallization with chloroform-methanol, it gave white needle shape crystals, m.p. 262-64°C (150 mg). Analysed for $C_{30}H_{50}O$: Calcd.: C, 84.50; H, 11.3%. Found:C, 84.47; H, 11.1%

^{1}H-NMR (200 MHz, $CDCl_3$) on δ-scale:

0.72 (3H, s, CH_3), 0.87 (3H, s, CH_3), 0.89 (3H, s, CH_3), 0.92 (3H, s, CH_3), 0.95 (6H, s, $2CH_3$), 1.05 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.25, 1.34, 1.45, 1.52, 1.58 (22 protons, m, -CH_2), 2.26-2.41 [3H, m, (C_2-2H and C_4-1H)].

IR, υ^{KBr}_{max} cm^{-1}:

2900 (CH, str), 1705 (>C=O), 1455, 1385, 1355, 1170, 1070.

Mass, m/z:

426 ($M^{+\bullet}$, 92%), 411 (40), 341 (32), 303 (70), 273 (100).

Compound (4):

Compound **(4)** obtained by elution of the column with petrol-benzene (2:3) and crystallized as white needles from chloroform-methanol, m.p. 198°C, R_f = 0.63 (benzene-chloroform, 8:2). It gave positive Liebermann-Burchard test.

^{1}H-NMR (100 MHz, $CDCl_3$) on δ-scale:

0.78 (3H, s), 0.83 (3H, s), 0.88 (6H, s), 0.95 (3H, s), 0.98(3H, s), 1.0 (3H, s), 1.14 (3H, s), 1.08, 2.01 (-CH_2 and -CH protons of cyclic system and side chain), 3.01 (1H, dd, J=9 Hz and 7 Hz), 4.88 (a broad singlet, 1H, OH proton), 5.21 (1H, m, olefinic proton).

IR, υ^{KBr}_{max} cm^{-1}:

3360 (OH), 2960, 2880, 1650, 1465 (C=C), 1040 and 980 cm^{-1}.

Mass, m/z:

426 $[M]^{+\bullet}$

Acetylation of Compound (4):

The compound **(4)** (25mg) was acetylated by heating it with acetic anhydride (1 ml) and pyridine (0.5 ml) on a boiling water bath for 4 hours. The reaction mixture was cooled at room temperature and poured over crushed ice. The solid obtained was washed well with water and dried. On crystallization from chloroform-methanol, it gave colourless needles m.p. 241-42°C, $[\alpha]_D^{23}$ + 68.9°.

IR, υ^{KBr}_{max} cm^{-1}:

1722 and 1240 (OAc), 1635, 812.

^{1}H-NMR ($CDCl_3$) on δ-scale:

0.84 (3H, s, H-28), 0.89 (12H, s, H-23, 24, 29, 30), 0.96 (6H, s, H-25, 26), 1.14 (3H, s, H-27), 2.08 (3H, s, OAc), 4.54 (1H, dd, J=6 Hz, H-3α), 5.20 (1H, t, J=3.5 Hz, H-12).

Compound (5):

Elution of the column with petrol-benzene (3:7) gave a TLC homogeneous substance which on repeated crystallization from chloroform-ethanol afforded white needle shaped crystals (70 mg) m.p. 136-37°C. It gave an acetate (Ac_2O/py), m.p. 114-15°C, $[\alpha]_D$ – 48.5° ($CHCl_3$) and monobenzoate, m.p. 145-46°C.

IR, υ^{KBr}_{max} cm^{-1}:

3340 (OH), 1055, 1655, 840 (C=C), 1460, 1375 (C-Me_2).

^{1}H-NMR (90 MHz $CDCl_3$) on δ-scale:

0.70 (3H, s, 18-Me), 0.80 (3H, d, J=6.8 Hz, 28-Me), 0.88 (6H, d, J=6.5 Hz, 26, 27-Me), 0.92 (3H, d, J=6.5 Hz, 21-Me), 1.02 (3H, s, 19-Me), 3.56 (1H, m, 3-ax-H), 5.36 (1H, m, olefinic proton), 1.07-2.34 (-CH_2 and –CH protons of cyclic system side chain).

Mass, m/z:

414 $[M]^{+\bullet}$

Acetylation of Compound (5):

Crystalline **(5)** (30 mg) was treated with acetic anhydride (2 ml) and pyridine (1 ml) and allowed to stand overnight at room temperature and then heated on a steam bath for 2 hours. After usual work up the solid was washed well with water and dried. On several crystallization from chloroform-methanol it gave colourless flakes (15 mg), m.p. 114-15°C $[\alpha]^{17}_D$ – 48.5°.

Analysed for $C_{31}H_{52}O_2$: Calcd.: C, 81.57; H, 11.40%. Found: C, 81.52; H, 11.37%

IR, υ^{KBr}_{max} cm^{-1}:

2930, 2850, 1730, 1660, 1460, 1380, 1260, 960.

Benzoate Formation:

The **compound (5)** (40 mg) was treated with benzoyl chloride (1 ml) and pyridine (0.5 ml), the mixture was allowed to stand at room temperature overnight and then heated for about 6 hours on a water bath. The reaction mixture was cooled and ice-cold water was added. The solid thus separated was filtered, washed with aqueous solution of potassium hydroxide (KOH) (2%) and then with water. It was crystallized from methanol, m.p. 145-46°C (25 mg), $[\alpha]_D^{17}$-7.52°.

Compound (6):

The benzene-ethylacetate fractions (1:1) of the column were found to be identical on TLC and therefore pooled together. Recovery of the solvent gave a gummy mass. On TLC examination it showed the presence of one major compound along with some minor impurities which were removed by crystallization with benzene-acetone, and yellow shining crystals of **(6)** were obtained m.p. 352°C. Analysed for $C_{15}H_{10}O_5$: Calcd.: C, 66.66; H, 3.70%. Found: C, 66.78; H, 3.74%

UV with shift reagents, λ_{max} nm: MeOH 265,297 sh, 338

$AlCl_3$	279, 300, 340, 390
$AlCl_3$/HCl	279, 299, 340, 389
NaOAc	279, 304, 376
NaOAc/H_3BO_3	266, 300 sh, 338.

Acetylation of Compound (6):

Crystalline **(6)** (25 mg) was treated with acetic anhydride (2 ml) and pyridine (1 ml) and

allowed to stand overnight at room temperature and then heated on a water bath for 2 hours. After usual work up as described earlier the solid obtained on several crystallization from chloroform-methanol gave colour-less crystals m.p. 183-84°C.

^{1}H-NMR (100 MHz, $CDCl_3$) on δ-scale:

7.85 (2H, d, J=9 Hz, H-2',6'), 7.04 (2H, d, J=9 Hz, H-3',5'), 6.60 (1H, s, H-3), 6.66 (1H, d, J=2.5 Hz, H-8), 6.51 (1H, d, J=2.5 Hz, H-6), 2.42 (3H, s, OAc-5), 2.35 (6H, s, OAc-4',7).

Compound (7):

Compound (7) was obtained from the fractions obtained by elution of the column with benzene-ethylacetate (1:1). On repeated crystallization with ethylacetate-acetone afforded yellow fine crystals m.p. >315^{0}C were obtained.

Analysed for $C_{15}H_{10}O_6$: Calcd.: C, 62.93; H, 3.49%.Found: C, 62.94; H, 3.50%

IR, υ^{KBr}_{max} cm^{-1}:

3400 (OH), 1640 (>C=O), 800, 840

UV with shift reagents, λ_{max} nm:

MeOH	258, 265, 292 sh, 346
NaOMe	296, 328 sh, 396
$AlCl_3$/HCl	267 sh, 295 sh, 355, 384
NaOAc	291, 326 sh, 377
NaOAc/H_3BO_3	277, 291 sh, 360, 432 sh

Acetylation of Compound (7):

Crystalline **(7)** was treated with acetic anhydride (2 ml) and pyridine (1 ml) and allowed to stand overnight at room temperature and then heated on a water bath for 2 hours. After usual work up, it was crystallized with chloroform-methanol as colourless needles m.p. 200-201°C.

^{1}H-NMR (270 MHz, $CDCl_3$) on δ-scale:

6.45 (1H, d, J=2.5 Hz, H-6), 6.59 (1H, s, H-3), 6.95 (1H, d, J=2.5 Hz, H-8), 7.25 (1H, d, J=9 Hz, H-5'), 7.75 (1H, dd, J_1=9 Hz & J_2=2.20 Hz, H-6'), 7.80 (1H, d, J=2.20 Hz, H-2'), 2.43 (3H, s, OAc-5), 2.35 (3H, s, OAc-7), 2.33 (6H, s, OAc-3',4').

Mass, m/z:

286 $[M]^{+\bullet}$, 153 $[A_1+H]^{+\bullet}$, 134 $[B_1]^{+\bullet}$

Compound (8):

It was obtained by elution of column with benzene-ethylecetate (1:1) and was crystallized from methanol as yellow crystals m.p. 311-12^{0}C. Analysed for $C_{15}H_{10}O_7$: Calcd.: C, 59.62; H, 3.31%.Found:C, 59.70; H, 3.33% **UV with shift reagents, λ_{max} nm:**

MeOH	256, 270 sh, 301 sh, 372
NaOMe	247 sh, 321 (Dec)
$AlCl_3$	274, 304 sh, 334, 458
$AlCl_3$/HCl	264, 358, 427
NaOAc	257 sh, 274, 329, 390
NaOAc/H_3BO_3	264, 303 sh, 389

Acetylation of Compound (8):

Crystalline **(8)** (20 mg) was acetylated by heating it with acetic anhydride (2 ml) and pyridine (1 ml) and allowed to stand overnight at room temperature and then heated on a water bath for 2 hours. The reaction mixture was cooled at room temperature and poured on crushed ice. The solid was collected, washed with water and dried. On crystallization from methanol, it gave cream coloured m.p. 194-95°C (10 mg).

^{1}H-NMR (100 MHz, $CDCl_3$) on δ-scale:

7.74 (1H, d, J=2.5 Hz, H-2'), 7.63 (q, J_1=2.5 Hz, J_2=8.5 Hz, H-6'), 6.92 (1H, d, J=8.5 Hz, H-5'), 6.87 (1H, d, J=2.5 Hz, H-8), 6.65 (1H, d, J=2.5 Hz, H-6), 2.35-2.40 (15H, m, 5 x OAc).

Compound (9):

Elution of the column with ethylacetate gave a fraction which on crystallization with methanol afforded pale yellow crystals m.p168^{0}C yield (30 mg). It was analysed for $C_{23}H_{18}O_4$.

IR, υ^{KBr}_{max} cm^{-1}:

2980 (br, OH), 1670, 1480 (C=C)

UV with shift reagents, λ_{max} nm:

MeOH	262, 295, 339
NaOMe	267, 338 (Dec)
$AlCl_3$	272, 299, 367
$AlCl_3$/HCl	273, 369
NaOAc	273, 324
NaOAc/H_3BO_3	269, 296 (Dec)

^{1}H-NMR (200 MHz, DMSO-d_6) on δ-scale:

2.50 (3H, s, CH_3), 2.59 (2H, s, CH_2), 6.17 (1H, d, J=2.5 Hz, H-6), 6.40 (1H, d, J=2.5 Hz, H-8), 6.89 (4H, d, J=9 Hz and 2.5 Hz, H-3',5',3",5"), 7.56 (4H, d, J=9 Hz and 2.5 Hz, H-2',6',2"',6"), 7.86 (1H, s, H-2), 2.59 (2H, s, CH_2), 9.27 (1H, brs, 7-OH), 12.46 (1H, s, 5-OH).

Mass, m/z:

358 ($M^{+\bullet}$) (3.2%), 314 ($M^{+\bullet}$-Co-CH_3-H, 15%), 326 ($M^{+\bullet}$-H_2O-CH_3+H, 10%). RDA fragment 152 (2%), 124 (152-CO, 10%), 91 (100%), 206 (18%).

Compound (10):

Compound (10) was crystallized from methanol-chloroform mixture as yellow needles m.p. 263-64°C (250 mg). It gave dark reddish-colour with Mg-HCl, and positive Molish test (2 ml of aq. extract of the compound was added two drops of a freshly prepared 20% alcoholic solution of α-naphthol. The mixture on treatment with 2 ml of conc. H_2SO_4 produced a red-violet ring which disappeared on the addition of an excess of alkali solution) and a dark

brown colour with alcoholic $FeCl_3$ solution.

Analysed for $C_{21}H_{20}O_{10}$:

Calcd: C, 56.12; H, 4.67%

Found: C, 56.09; H, 4.62%

IR, υ^{KBr}_{max} cm^{-1}:

3420(OH), 2950,1650(C=O)

UV with shift reagents, λ_{max} nm:

MeOH	268, 298, 335
NaOMe	278, 325, 393
$AlCl_3$	279, 301, 353, 384
$AlCl_3$/HCl	276, 300, 351, 385
NaOAc	278, 300, 377
NaOAc/H3BO_3	270, 308 sh, 342

Acetylation of Compound (10):

The crystalline glycoside **(10)** (30 mg), was dissolved in pyridine (1 ml) and acetic anhydride (2 ml) was added. The mixture was heated on a water bath for about 3 hrs. and then left overnight at room temperature. After usual work up, the solid obtained was crystallized from ethylacetate-petroleum ether as white crystals (At-10Ac), m.p. 154-56°C.

Analysed for $C_{33}H_{32}O_{16}$: Calcd:C, 57.89; H, 4.67% found: C, 57.79; H, 4.63%

^{1}H-NMR (300 MHz, $CDCl_3$) on δ scale:

1.72-2.02 (12 H, m, 4 x OAc, aliphatic acetoxyls), 2.32, 2.43, 2.51 (9H, s, 3 x OAc, aromatic acetoxyls),3.65-5.70 (7H, m, H-1",2",3",4",5",6"), 4.64 (1H, d, J=10 Hz, H-1"), 6.8 (1H, s, H-3), 6.91 (1H, s, H-6), 7.4 (2H, d, J=9 Hz, H-3', 5'), 8.1 (2H, d, J=9 Hz, H-2',6').

Hydroiodic acid oxidation:

A mixture of the glycoside **(10)** (30 mg), phenol (70 mg) and hydroiodic acid (0.3 ml) was refluxed for about 9 hours. The mixture was cooled and sodium bisulphite ($NaHSO_3$) was added to it with stirring. The separated brown substance was purified by passing it through a silica gel column.

Elution of the column with benzene-ethylacetate (1:1) mixture afforded the substance, At-10agl. It was crystallized from chloroform-ethylacetate as light-yellow crystals. Yield (15 mg), m.p. 347-48°C. Analysed for $C_{15}H_{10}O_5$:

Calcd: C, 66.66; H, 3.70%

Found: C, 66.64; H, 3.54%

UV data with shift reagents, λ_{max} nm:

MeOH	266, 297, 336
NaOMe	277, 326, 395
$AlCl_3$	280, 303, 351, 382
$AlCl_3$/HCl	278, 300, 352, 384
NaOAc	276, 300, 378
NaOAc/H_3BO_3	270, 306 sh, 345

Ferric chloride oxidation:

The glycoside **(10)** (30 mg) was added to a solution of $FeCl_3$ (120 mg) in 3 ml water. The mixture was heated on an oil bath at 125°C for about 7 hours. The reaction mixture after cooling, was diluted with water (10 ml), a small amount of dark coloured solid formed was filtered off. The filtrate was purified by passing through a column of silica gel using water as eluant. The initial fractions obtained were combined and concentrated to a syrup which was subjected to paper chromatography on Whatman No. 1 filter sheet using n-BuOH-AcOH-H_2O (4:1:5) and n-BuOH-water-ethanol (60:25:8:16.5) as solvent systems and employing the descending technique. Authentic sugars were used as checks. The chromatograms were run for 24 hrs. and after drying at room temperature were sprayed with aniline phthalate and p-anisidine phosphate solutions. The chromatograms on drying at 100-105°C revealed the presence of glucose only.

GLC of Trimethyl silyl ether of sugar:

The TMSi ether of sugar was prepared by taking 15 mg of sugar in dry pyridine (0.5 ml) and hexamethyl disilazane (0.2 ml) in a 10 ml round bottom flask. To this solution 0.2 ml of trimethyl chlorosilane was added. The flask was stoppered and kept at room temperature for one hour. The solution after drying was taken in heptane. The heptane soluble TMSi ether derivative of sugar was then subjected to **glc** (2% OV-1, column temp. 150-250°C, 10 min. dect. temp. 300°, N_2, 50 ml/min) along with the silyl derivative of standard sugar. The observed Rt. value was found to be in agreement with that of authentic sample of glucose (Rt. glucose 1.0).

Periodate oxidation of glycoside methyl ether:

Glycoside methyl ether (15 mg) of **(10)** was dissolved in methanol (10 ml) and an aqueous solution of $NaIO_4$ (0.47 N, 15 ml) was added to it. The mixture was kept at 20°C in dark for 24 hours. Solid $NaHCO_3$ (2 gm) was then added followed by the addition of Na_3AsO_3 solution (0.05 N, 25 ml). The resultant mixture was titrated against iodine using starch as indicator. One mole of methyl ether consumed 1.2 mole of periodate, with the liberation of one mole of formic acid

Compound (11):

Fractions obtained by the elution of the column with ethylacetate-methanol (8:2-7:3) gave pale yellow solid. It was crystallized from methanol-chloroform as pale-yellow crystals (160 mg), m.p.

>280. Analysed for $C_{21}H_{22}O_9$: Calcd: C, 60.28; H, 5.26%Found: C, 60.23; H, 5.20%

IR, υ^{KBr}_{max} cm^{-1}: 2965 (chelated OH), 1684 (C=O), 1462 (C=C)

UV data with shift reagents, λ_{max} nm:

MeOH	245, 278, 322 sh, 365
NaOMe	244, 271, 324 sh, 366
$AlCl_3$	246, 284, 320 sh, 435
$AlCl_3$/HCl	246, 282, 325 sh, 436
NaOAc	245, 277, 324 sh, 366
$NaOAc/H_3BO_3$	245, 276, 323 sh, 365

^{1}H-NMR (300 MHz, DMSO) on δ scale:

6.24 (1H, d, J=2.0 Hz, H-3'), 6.26 (1H, d, J=2.0 Hz, H-5'), 6.77-6.85 (5H, m, H-2,3,4,5,6), 6.97 (1H, d, J=15 Hz, H-α), 7.85 (1H, d, J=15 Hz, H-β), sugar protons: 4.1 (1H, d, J=9 Hz, H-1"glu), 3.5-4.32 (4H, m, H-1",2",3",4"), 5.22-5.50 (3H, m, H-5",6"), 13.60 (1H, s, 2'-OH), 12.85 (1H, s, 6'-OH).

Mass, m/z:

$M^{+\bullet}$ 418 (18%), 256 (10.4%), 238 (6.4%), 228 (6.8%), 104 (8.5%), 103 (15%), 152 (10.8%), 179 (6.3%), 131 (7.4%), 91 (10%), 126 (16.4%), 55 (7.8%), 54 (7.5%), 78 (6%), 180 (10.6%).

Acetylation of Compound (11):

Compound (11) (30 mg), was dissolved in pyridine (1 ml) and acetic anhydride (2 ml) was added. The mixture was heated on a water bath for about 3 hrs. After usual work up, as described earlier, the acetate obtained was crystallized from chloroform-methanol as fine needle sharped crystals (15 mg), m.p. 178^{0}C.

^{1}H-NMR (200 MHz, $CDCl_3$) on δ scale:

1.78-2.05 (12H, m, 4 aliphatic acetoxyls), 2.52 (6H, s, aromatic acetoxyls OAc-2',6'), 6.24 (1H, d, J=2.0 Hz, H-3'), 6.26 (1H, d, J=2.0 Hz, H-5'), 6.77-6.85 (5H, m, H-2,3,4,5,6), 6.97 (1H, d, J=15 Hz, H-α), 7.85 (1H, d, J=15 Hz, H-β), 4.1 (1H, d, J=9 Hz, H-1" glu), 3.5-4.32 (4H, m, H-1",2",3",4"), 5.22-5.50 (3H, m, H-5",6").

Acid Hydrolysis of Compound (11):

The glucoside **(11)** (60 mg) was hydrolysed by heating with 6% aqueous HCl on a water bath. The heating was continued for 2 hrs to ensure complete hydrolysis. The mixture was left overnight. The aglycone which settled down was filtered, washed with water and dried. It was crystallized with methanol-chloroform as yellow crystals yield (35 mg) m.p 176^{0}C.

^{1}H-NMR (DMSO) on δ scale:

6.20 (H, d, J=2.0 Hz, H-3'), 6.23 (1H, d, J=2.0 Hz, H-5'), 6.75-6.82 (5H, m, H-2,3,4,5,6), 6.95 (1H, d, J=15 Hz, H-α), 7.81 (1H, d, J=15 Hz, H-β), 13.0 (1H, s, 2'-OH), 12.50 (1H, s, 2'-OH), 10.1 (1H, s, 4'-OH).

UV data with shift reagents, λ_{max} nm:

MeOH	251, 298 sh, 368
NaOMe	253, 280 sh, 319 sh, 346 sh, 430
$AlCl_3$	255, 321, 385 sh, 423
$AlCl_3$/HCl	319 sh, 378 sh, 421
NaOAc	282 sh, 341, 350 sh, 392
$NaOAc/H_3BO_3$	286, 353 sh, 381, 443

Acetylation of aglycone:

Aglycone (12 mg) was acetylated by heating with pyridine (1 ml) and acetic andydride (2 ml) over water bath for 3 hours. After usual work up it was crystallized with chloroform-methanol as white needles, m.p 155-56^{0}C.

^{1}H-NMR ($CDCl_3$) on δ scale:

2.45 (6H, s, OAc-2',6'), 2.34 (2H, s, OAc-4'), 6.22 (1H, d, J=2.0 Hz, H-3'), 6.25 (1H, d, J=2.0 Hz, H-5'), 6.78-6.83 (5H, m, H-2',3',4',5',6'), 6.99 (1H, d, J=16.0, H-β).

Degradation of aglycone:

The aglycone (10 mg) was dissolved in 50% KOH (2 ml). The mixture was heated over water bath for 3 hrs. it was cooled and acidified by HCl. The solution was extracted with ether. And ether layer was washed with water to remove excess of HCl, then was shaken by $NaHCO_3$ solution, aqueous and organic layers were separated. The ether was dried by passing over anhydrous sodium sulphate and evaporated, the residue on co-TLC examination with an authentic sample of pholoroglucinol showed it to be pholoroglucinol.

The aqueous layer was acidified by adding HCl and extracted with ether. The ether layer was washed with water and dried with sodium sulphate. The identity of the residue was checked by TLC and co-TLC with an authentic sample of cinnamic acid.

Identification of Sugar:

The hydrolysate was concentrated and neutralized over KOH under vacuum and chromatographed on Whatman (No.1) filter paper using n-butanol-acetic acid-water (4:1:5 v/v/v) as the developing system, employing the descending technique. Authentic sugars were used as checks. The chromatogram was run for 24 hours, after developing, it was dried at room temperature and sprayed with aniline hydrogen phthalate solution heated at 100-5^{0}C for 10 minutes.

The sugar was identified as glucose (R_f 0.18) by comparison with authentic sugar (R_f, co-paper chromatography).

Estimation of Sugar:

The anhydrous glucoside (30 mg) was hydrolysed by refluxing with 2% H_2SO_4 for 2 hours. After cooling overnight, the aglycone was filtered and dried. The ratio of the aglycone to the glycoside was found to be (1:2) indicating the presence of 1 mole of sugar per mole of the aglycone.

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