Pluchea Cass. (Compositeae) A genus of shrubs or under-shrubs, rarely herbs, distributed in the tropical and subtropical regions of the world. Six species have been recorded in India.

P. indica Less. Beng.-Kukronda, munjhu rukha. A low shrub found in salt marshes and mangrove swamps in Sunderbans. Leaves 2-5 cm. long, obovate or oblanceolate, sub-serrate, narrowed into a short petiole; flowers lilac, in heads in terminal corymbs.

The plant is drought resistant and grows on heavy soils. The leaves flowers and young tops are eaten raw or cooked. In Thailand and Java, the aromatic leaves are used as a flavouring. Leaves contain 2.9 percent protein. The plant contains chlorogenic acid (uphof 287, Burkill, II, 1733; Terra, Commun. R. Trop. Inst. Amst., No. 54 e, 1966, 68, Wehmer, II, 1213).

The roots and leaves are reported to possess astringent and antipyretic properties and are given in decoction as a diaphoretic in fevers. Leaf juice is taken for dysentery in Malaya. In Indo-China, an infusion of leaves is given for lumbago; it is also used as a remedy against leucorrhoea. The leaves are used in baths (as nervine tonic), in poultices against atonic and gangrenous ulcers. In Malaya, the leaves are used as a constituent in lea, for slimming. Recently, a hybrid of this species with P. odorata Cass. has been reported; the latter is a medicinal plant of Central America (Kirt & Basu, II, 1345; Burkill, II, 1773; Van Steenis-Kruseman; Bull. Org. Sci. Res. Indonesia, No. 18, 1953, 19; Hoppe, 714; Cooperrider & Galang, Amer. J. Bot. 1965, 52, 1020).
Pluchea lanceolata
**P. lanceolata** C.B. Clarke Hindi, Mar. & Guj- Rasana, rashana, Punjab- Sarmei, reshami, Uttar Pradesh- Baisurai; Rajasthan- Chotakalia Delhi-Rukhri. An erect undershrub, 30-100 cm. tall, found in sandy or saline soils in Punjab Upper gangetic plain, Rajasthan and Gujarat. Leaves 2-6 cm. long, sessile, oblanceolate or oblong, coriaceous; flowers white yellow, lilac or purple, in many headed compound corymbs.

**P. lanceolata** occurs gregriously in vast areas in forming thickers and is considered a troublesome weed. It does much damage to rabi crops, particularly in areas where irrigation facilities are not available. The plant is succulent when young, with sufficient foliage, and on this account, it has been tried as a possible cattle fodder in some of the drier parts of Uttar Pradesh cattle, however, avoid it when grazing, because of its peculiar disagreeable bitter taste. It can be fed only to working cattle either in mixture with bhusa or jowar stalks. Analysis show a fairly high percentage of protein and a much greater feeding value than bhusa or jowar stalks. Bullocks fed on baisurai-jowar mixture show a sleek well-fed appearance (Parr and Dayal, Agric. J. India, 1921, 16, 106; Parr. & Lal, ibid, 1921, 16, 206).

As a trouble some weed, it can be eradicated by spraying Agroxone (10%) or Fernoxone (2,4-D) (Shivapuri and Tyagi, Indian Fund., 1950, II, 116; Tandon, Agric. Anim. Husb., Uttar Pradesh, 1951, I(9), 9).

The leaves are succulent and are considered aperient; they are used as substitute or adulterant for senvac. The plant is mentioned in Ayurvedic texts to be used in diseases similar to rheumatoid arthritis. A decoction of the plant has been reported to prevent the swelling of joints in experimental
arthritis. Preliminary studies on the plant revealed the presence of glycoside and sterol. Pharmacological investigations indicated that the drug had two primary actions, viz. acetylcholine-like action and smooth muscle relaxant-spasmolytic action on different muscle preparations. The only central nervous system activity detected in the drug was that of potentiation of barbiturate hypnosis. In a recent investigation, quercetin and iso rhamnetin were identified in the air dried leaves, glycosides were absent (Singh, Indian j. Agric. Sci. 1945, 15, 297; Kirt & Basu, II, 134; Chaturvedi & Singh, Indian J. Med. Res. 1965, 53, 71; Prasad et al., ibid, 1966, 54, 582; 1965, 53, 1062; Bahl et al., Curr. Sci. 1968, 37, 1).

Previous Work

No chemical work has been reported in literature from Pluchea lanceolata but some work has been reported on other species of Pluchea. Sesquiterpenes have been isolated from Pluchea chingoyo D.C. Plucheinot and cuanhtemone were also obtained. Naturally occurring terpene derivatives, a new eudesmane derivatives from P. naveolens and P. foetida were obtained. Anticancer agents from P. chingoyo D.C. were isolated. P. sagitallis is a composite growing in low humid regions of Argentina, Brazil, Uruguay and Paraguay. Essential oil, terpenoid were isolated. The 4 novel dehydroendesmanones were isolated from the leaves of P. odorata. Three endesmanolides from P. rosea have been isolated. Some essential oils have been isolated from P. fastigiata, P. purpurescens, P. salicifolia.
Present Work

The dried and powdered plant material of *Pluchea lanceolata* Linn. was extracted with petrol ether, ethyl alcohol and aqueous ethanol.

**Constituents of Petrol ether extract**

The petrol ether extract was concentrated and kept in cold. A semi solid mass was separated, which was filtered and subjected to column chromatography over alumina using petrol ether, benzene, chloroform and methyl alcohol as successive eluents. A number of compounds were separated which were worked out as below.

**Compound A**

The first compound was crystallized from alcohol furnishing fine needles, $C_{36}H_{60}O_2$; mp. 158-160°. The IR spectrum had absorption bands at $1725$ cm$^{-1}$ for the ester carbonyl supported by a band at $1250$ cm$^{-1}$ besides the usual absorption bands. The mass spectrum of the compound showed peaks at m/e $524$ (M$^+$), $509$ (M$^+$ - CH$_3$), $408$ (M$^+$ - C$_5$H$_{11}$ COOH), 218, 203 & 189. The compound was identified as β-amyrin caproate on the basis of mass fragmentation pattern, superimposable IR spectra and CO-TLC.

**Compound B**

The hexane: benzene elutions of the column on concentration and crystallization gave a crystalline white compound mp. 156-158°. Chloroformic solution of the compound showed Liebermann-Burchardt test positive. The benzoylation of α-amyrin gives the mp. 179-180° (α) D + 97°. Debenzoylation of the compound gives mp. 180-182°; (α) D + 82°; max 3450 cm$^{-1}$, MS
m/e 426, 411, 393, 218, 207, 203 & 189. The compound was finally charac-
terised as α-amyrin by mixed melting point CO-TLC, superimposable IR and
Mass fragmentation with an authentic sample.

Compound C

The benzene eluate on concentration and crystallization with methanol
gave white crystalline compound mp. 171° (α) D=51.0° analysed for C_{29}H_{48}O.
Mass spectrum m/e 412\(^{+}\) with other peaks at m/e 397 (M-CH\(_3\)), 394 (M-HOH)
369, 351, 300, 271 (M-Side chain -2H), 255 (M-side chain HOH). The mono-
acetate prepared by pyridine and acetic anhydride in the usual manner after
crystallization melted at 139° analysed for C_{31}H_{50}O_{2}. The compound was
finally identified to be stigmasterol through mixed mp. and superimposable
IR with an authentic sample.

Constitutents of Alcohol extract

The alcohol extract of the plant material was concentrated under reduced
pressure and successively macerated with petrol ether and chloroform and
the residual alcoholic extract was hydrolysed and the aglycones thus obtained
were subjected to column chromatography over silica gel affording different
compounds.

Compound D

The chloroform eluents gave a shinoda test positive yellow colouring
matter. Its UV absorption spectra in methanol showed peaks at 253sh, 266,
294sh, 322sh, 367 nm. Addition of aqueous alkali showed bathochromic shift
confirming the presence of hydroxyl group. On addition of NaOAc the spectrum
showed bathochromic shift (266-274) which indicates the presence of 7-OH group. No appreciable shift was observed with NaOAc/H$_3$BO$_3$ which indicate absence of ortho OH group. The methanolic solution with aluminium chloride showed a bathochromic shift at 56 nm in the longer wave length indicating the presence of another OH group in 3 position$^{3,4}$. On addition on NaOMe the longer wavelength peaks disappear and peak at 416 nm appear indicating the presence of 3,4' OH groups. The IR spectrum showed peaks at 3450 cm$^{-1}$ (OH) and 1660 cm$^{-1}$ (CO). Rf value was found to be 0.81 in BAW (6:1:2) and 0.04 in 15 percent acetic acid. From the UV, IR and Rf data it appears the compound is a flavonoid having phenolic hydroxy group at 3', 5, 7 and 4' positions. From the above spectral analysis the compound was identified as kaempferol$^{15}$, the identity of which was further confirmed through mixed mp. CO-TLC and superimposable IR with an authentic sample.

Compound E

The chloroform: ethyl acetate eluate on concentration gave a shinoda test positive yellow colouring matter. The UV absorption in methanol showed characteristic bands at 255, 258sh, 202sh, and 371 nm. Addition of alkali produces a bathochromic shift. On addition of fused NaOAc the spectrum showed a bathochromic shift (255-274) and NaOAc/H$_3$BO$_3$ produces a shift of longer wavelength (377-388 nm). The IR spectrum showed peaks at 3500 (OH) and 1660 (CO). The UV and IR spectrum and Rf value in different solvent system showed compound to be quercetin the identity of which was finally confirmed through mixed mp. CO-TLC and superimposable IR with an authentic sample.
U.V. SPECTRUM OF ISORHAMNETIN

\[ \text{MeOH} \]
\[ \text{MeOH} \cdot \text{NaOMe} \]

\[ \text{MeOH} \cdot \text{AlCl}_3 \]
\[ \text{MeOH} + \text{HCl} \]

\[ \text{MeOH} \cdot \text{NaOAc} \]
\[ \text{MeOH} \cdot \text{NaOAc} + \text{H}_3\text{BO}_3 \]
Compound F

The ethyl acetate eluate on concentration gave dull yellow colour in UV light and in UV/NH$_3$ the same colour. UV spectral data in Methanol 253, 267sh, 306sh, 370 nm and in NaOMe 240sh, 271, 328, 435 nm. On addition of fused NaOAc the spectrum showed a bathochromic shift 260sh, 274, 320, 393 nm and NaOAc/H$_3$BO$_3$ produces a shift of layer wavelength 255, 270sh, 306sh, 326sh, 377 nm. The IR spectrum showed peaks at 3500 (OH) and 1660 (CO). Rf values in paper chromatography 0.68 (TBA), 0.02 (HOAc). The UV, IR spectrum and Rf values in different solvent system showed compound to be Isorhamnetin$^{17}$, the identity of which was finally confirmed through mixed mp., CO-TLC and super imposable IR with an authentic sample.

Constituents of aqueous ethyl alcohol

The defatted plant material was extracted with 70% ethanol. The extract was concentrated and extracted with ethyl acetate. Paper chromatography$^{18}$ of the ethyl acetate extract revealed the presence of four fluorescent spots named F, G, H & I when observed under long range UV light. Flavonoid separation was carried out in Whatman No. 3 mm paper using Butanol: acetic acid: water (3:1:1); 15 percent acetic acid as solvent system. Individual compounds were isolated by means of paper chromatography using IDPC and 2 DPC technique. Concentrate of the solvent extract were applied to several 3 MM sheet and band observed in high concentration under UV light were cut and eluted with methanol purified by paper and column chromatography.

Compound G

The pigment precipitated as the lead salt by the addition of neutral and basic lead acetates in alcohol extract. The major basic lead acetate
fraction is a glycoside yielding on hydrolysis kaempferol, glucose and rhamnose. Its colour reactions indicated that this was a 3 glycoside.

The UV spectral data of the compound in methanol showed band at 267, 345 nm. The absence of a maximum at 310 nm indicated that the 3-hydroxy group is not free. Complete methylation and hydrolysis confirmed this inference since the product was kaempferol trimethyl ether with the 3-hydroxyl free. UV absorption in ethanol 269, 305, 356 nm in sodium ethoxide 277, 332 and 410 nm. Hydrolysis of the glycosides with 7 percent H₂SO₄ yielded a small amount of the aglycone which was identified as kaempferol; the sugars were found to be glucose and rhamnose by their Rf values and by CO-chromatography with pure sample. The compound is kaempferol-3-rutinoside confirmed by UV and CO-chromatography.

**Compound H**

The UV spectral data of the compound in methanol showed band at 259, 266sh, 299sh, 359 nm. 12 nm hypsochromic shift compared to quercetin shows 3-OH substituted. In addition of 2 drops 2 M NaOH showed band at 272, 327, 415 nm. Spectral effect 52 nm bathochromic shift showed 4'-OH free. In addition with AlCl₃ the bands are 275, 303sh, 433nm. In this 70 nm bathochromic shift (5-OH free). When added NaOAc the bands are 271, 325, 393nm. Spectral effect 12 nm shift in band 1 (7-OH free).

In addition of NaOAc and H₃BO₃ the spectral maxima 262, 298, 387 nm the spectral effect 20 nm bathochromic shift. (3,4'-di-OH) Rf values in paper chromatography 0.45 (BAW), 0.23 (water) and 0.51 (15 percent acetic acid)
colour in UV light dull brown and in UV + NH₃ is bright yellow. On hydrolysis this compound gives quercetin, glucose and rhamnose. The UV spectrum of quercetin in ethanol 255, 374 nm and the colour in UV and UV + NH₃ is yellow, Rf values 0.41 (forestal); 0.64 (BAW); 0.29 (PhOH). On the above spectral data the compound is Quercetin-3-rutinoside. The final identity of the compound was confirmed by UV and CO-chromatography of an authentic sample of Quercetin-3-rutinoside₁⁹.

**Compound I**

Dark UV absorbing spot turning green in NH₃. \( \lambda_{\text{max}} \) (MeOH) 271, 333 nm; (NaOMe) 282, 329, 398 nm, (NaOAc) 280, 301sh, 387 nm; (NaOAc-H₃BO₃) 273, 280sh, 320sh, 320sh. Rf values 0.28 (TBA), 0.50 (HOAc) 0.45 (BAW). The compound was isolated by preparative 2-DPC, Hydrolysis, 5% HCl, 100°, 6 hr left it paper chromatographically unchanged. The compound CO-chromatographed on paper (TBA and HOAc) and polyamide (TLC, MeOH-HOAc-H₂O 18:1:1), Rf 0.60, with vicenin-2 but not with violanthin. The compound is an apigenin-6-3-di-C-glicoside²⁰.

**Experimental**

The melting points (uncorrected) were determined in a gallenkemp melting point apparatus; unless stated otherwise, optical rotations were measured in chloroform at 1 percent concentration; IR spectra were recorded in KBr: UV spectra were taken in ethanol.
\[
\begin{align*}
\text{\(\beta\)-Amyrin caproate} & : \quad \text{CH}_3(\text{CH}_2)_4\text{OCO} \\
\text{\(\alpha\)-Amyrin} & : \quad \text{HO} \\
\text{Stigmasterol} & : \quad \text{HO} \\
\text{Kaempferol} & : \quad \text{HO} \\
\text{Quercetin} & : \quad \text{HO}
\end{align*}
\]
Alumina (S. Merck) and Silica gel (B.D.H.) were activated at 120° before use in the chromatographic columns. The solvents used for chromatography were moisture free. The course of reaction and the progress of column chromatography were followed by TLC. Thin layer chromatography was carried out on silica gel G (S. Merck) layers. The room temperature generally varied from 20-38° higher during summer months.

The dried and powdered plant material of Pluchea lanceolata Linn. was extracted with petrol ether, ethyl alcohol and aqueous ethanol.

**Constituents of Petrol ether extract**

The petrol ether extract was concentrated and kept in cold. A semi solid mass was separated, which was filtered and subjected to column chromatography over alumina using petrol ether, benzene, chloroform and methyl alcohol as successive eluants when the following crystalline compounds were obtained.

**Compound A-B-Amyrin caproate**

The elution of the column with hexane-benzene (9:1) gave a compound (0.15%) mp. 140-145°. The product on repeated crystallization from alcohol furnished fine needles mp. 158-160°; \( \nu \) max 2890, 1727, 1462, 1379, 1362, 1330, 1300, 1270, 1252, 1224, 1176, 827, 815 and 725 cm\(^{-1}\); MS, m/e 524, 509, 408, 393, 305, 218, 203 and 189. NMR \( \delta \) 4.84, 5.49, 7.83, 8.86, 9.02, 9.14 and 9.16.

Analysed for \( \text{found} \) C, 82.42; G, 11.43%  
C\(_{36}\)H\(_{60}\)O\(_2\) calcd. \( \text{C}, 82.40; \text{H}, 11.22\% \)
Hydrolysis of the ester:

The ester (250 mg) was refluxed with 5% alcoholic KOH (50 ml) at 100° for 3 hrs. The reaction mixture was poured on crushed ice. The precipitate thus obtained was filtered and washed acid free. The product on crystallization from alcohol furnished needles of β-amyrin, mp. 190-192; ν_max 3240 cm⁻¹ MS, m/e 426, 411, 393, 218, 207, 203 and 189.

Acetylation of β-amyrin

β-amyrin (50 mg) was treated with acetic anhydride (1 ml) and pyridine (1 ml) at room temperature and left overnight. After usual work up it yielded β-amyrin acetate (40 mg), mp. 232-234°, (α) D + 86°; ν_max 1735 and 1250 cm⁻¹ MS, m/e 468, 453, 408, 393, 249, 218, 203 and 189.

Analysed for found  C, 82.05; H, 11.11%
C₃₀H₅₂O₂  calcd.  C, 82.15; H, 11.32%

The aqueous filtrate was acidified with dil HCl and extracted with ether. The etherial layer was washed acid free, dried over anhydrous Na₂SO₄ and treated with ethereal diazomethane. The reaction mixture was left overnight washed with 1% aqueous KOH to remove unreacted product and finally with distilled water till alkali free and dried over anhydrous sodium sulphate. The methylated product, thus obtained on removal of the solvent was characterised as methyl caproate by CO-GLC with an authentic sample under following conditions.

Column: 10% EGSSX on Chromosorb W, 1.5 m x 4 mm.
Column temp: 155°
Injector temp: 170°
Detector temp: 180°
Carrier gas; Nitrogen at 50 ml/min.

**Compound B-\(\alpha\)-Amyrin**

The hexane-benzene elutions of the column furnished one more LB positive compound which on crystallization from alcohol gave fine needles mp. 156-158°. The compound was purified through its benzoate derivative.

**Benzoylation of \(\alpha\)-amyrin**

The compound (200 mg) in pyridine (2 ml) was treated with benzoyl chloride (2 ml) and the reaction mixture was allowed to stand at room temperature for 24 hours. After usual processing the resultant product (185 mg) was purified by column chromatography over neutral alumina. The hexane eluents of the column gave the product which was crystallized from alcohol mp. 179-180°; ( ) D + 97°.

Analysed for found C, 83.78; H, 10.22%

C\(_{37}\)H\(_{54}\)O\(_2\) calcd. C, 82.96; H, 10.52%

**Debenzoylation of the compound**

\(\alpha\)-amyrin benzoate (100 mg) was refluxed with 0.5 N alcoholic KOH (25 ml) for 3 hrs. on a steam bath. The reaction mixture was processed in usual manner and the product on crystallization afforded needles of \(\alpha\)-amyrin, (80 mg), mp. 180-182°, (\(\alpha\)) D + 82°; \(\nu\)max 3450 cm\(^{-1}\) MS m/e 426, 411, 393, 218, 207, 203 and 189.
Analysed for  

found  C, 84.42; H, 11.84%  

calcd.  C, 84.28; H, 12.05%  

α-Amyrin was finally characterized through mixed mp; CO-TLC, superimposable IR spectra, mass fragmentation pattern and acetate (Ac₂O/py) mp. 222-224⁰; (D + 82.5; ν_max 1730, 1370, 1361, 1241, 827, 818 and 804, cm⁻¹.

Analysed for  

found  C, 82.05; H, 11.11%  

calcd.  C, 81.90; H, 11.25%  

**Compound C-stigmasterol**

The benzene-eluate on concentration and crystallization from methanol gave white crystalline compound mp. 171⁰ (D = -51⁰).

Analysed for  

found  C, 84.28; H, 11.42%  

calcd.  C, 84.47; H, 11.65%  

Mass spectrum: m/e 412 (M⁺), 397 (M-CH₃), 394 (M-MOH), 369, 351, 300, 271 (M-side chain- 2H).

**Acetyl derivative of stigmasterol**

Stigmasterol in pyridine and acetic anhydride was kept over night and the reaction mixture after working up in the usual manner gave white mono-acetyl derivative crystallized from methanol, mp. 139⁰.

Analysed for  

found  C, 81.82; H, 11.12%  

calcd.  C,81.94; H, 11.01%  

**Compound D kaempferol**

The chloroform eluates gave a shinoda positive yellow compound.
UV data

\[ \text{Methanol max} \]
253sh, 266, 294sh, 322sh, 367 nm.

\[ \text{NaOAc max} \]
274, 303, 387 nm.

\[ \text{NaOMe max} \]
278, 316, 416 nm.

\[ \text{NaOAc/H}_3\text{BO}_3 \text{ max} \]
267, 297sh, 320sh, 372 nm.

\[ \text{AlCl}_3 \text{ max} \]
260 sh, 268, 303 sh, 350, 424 nm.

IR peaks
3450 cm\(^{-1}\) (OH), 1660 cm\(^{-1}\) (CO)

Mass: m/e 286 (M\(^+\))

Rf: 0.81-
Butanol:Acetic acid: Water (6:1:2)
0.04-
15 percent acetic acid.

Compound E- Quercetin

The chloroform: ethyl acetate eluate gave a shinoda positive compound crystallized from methanol mp., 305-308°.

UV spectral data

\[ \text{MeOH max} \]
255, 269 sh, 301 sh and 370 nm.

\[ \text{NaOAc max} \]
257 sh, 274, 329, 390 nm.

\[ \text{NaOAc/H}_3\text{BO}_3 \text{ max} \]
261, 303 sh, 388 nm.

IR spectrum
3500 cm\(^{-1}\) (OH), 1660 (CO).
Isorhamnetin

$R = \text{Rhamnoglucosyl}$  
$\text{Kaempferol-3-rutinoside}$

$R = \text{Rhamnoglucosyl}$  
$\text{Quercetin-3-rutinoside}$

$R = \text{Glucosyl}$  
$\text{Apigenin 6,8-di C-glucoside}$
Rf. of Quercetin in different solvent system

1. HCl: acetic acid : water (3:30:10) 0.41
2. Butanol: acetic acid : water (4:1:5) 0.64
3. Phenol : water (3 : 1) 0.29

Analysed for  
found  C, 59.52; H, 3.80%

C₁₅H₁₀O₇ calcd.  C, 59.60; H, 3.31%

It formed a penta acetate (Ac₂O/py) mp. 190-192°.

Analysed for  
found  C, 58.50; H, 3.05%

calcd.  C, 58.69; H, 3.90%

Quercetin was finally characterised through mixed mp. and paper CO-chromatography.

Compound F - Isorhamnetin

The ethyl acetate eluate on concentration gave dull yellow colour in UV light and in UV +NH₃ the same colour.

UV spectral data

MeOH max  253, 267sh, 306sh, 370 nm.
NaOMe max  240sh, 271, 328, 435 nm.
AlCl₃ max  264, 304sh, 361sh, 431 nm.
AlCl₃/HCl max  242sh, 262, 271sh, 302sh, 357, 428 nm.
NaOAc max  260sh, 274, 320, 393 nm.
NaOAc/H₃BO max  255, 270sh, 306sh, 377 nm.
IR spectrum: 3400 cm$^{-1}$ (OH), 1660 cm$^{-1}$ (CO)

Rf values of Rhamnetin in different solvent system

1. TBA - 0.68
2. Acetic acid - 0.02

Compound G - kaempferol-3-rutinoside

UV spectral data

<table>
<thead>
<tr>
<th>Solvent</th>
<th>λ$_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>267, 345 nm</td>
</tr>
<tr>
<td>Ethanol</td>
<td>269, 305, 356 nm</td>
</tr>
<tr>
<td>NaOEt</td>
<td>277, 332, 410 nm</td>
</tr>
</tbody>
</table>

Spot colour with Mg & HCl - Deep red
Spot colour with Zn & HCl - Violet pink
Spot colour with Alc. FeCl$_3$ - Greenish brown solution
Spot colour with Conc. H$_2$SO$_4$ - Yellow solution with blue fluorescence.

Hydrolysis of kaempferol-3-rutinoside

The glycoside (0.5 g) was hydrolysed by refluxing with 7 percent H$_2$SO$_4$ (50 ml) for 2 hr. After cooling, the yellow aglycone was filtered and the filtrate extracted with ether; it crystallized from methanol-ethermixture as pale yellow needle decomposing at 278-280°. It was identified as kaempferol by mixed mp, colour reactions, Rf values and CO-chromatography with standard specimen.
Rf values of the aglycone in different solvent system

1. Conc. HCl : acetic acid : water (3:30:10) - 0.55
2. nButanol: acetic acid: water (4:1:5) - 0.83
3. PhOH: Water (3:1) - 0.58

UV spectral data of the aglycone

\[
\text{methanol} \quad \lambda_{\text{max}} \quad 253\text{sh}, 266, 294\text{sh}, 322\text{sh}, 367\text{nm}.
\]
\[
\text{NaOAc} \quad \lambda_{\text{max}} \quad 274, 303, 387\text{ nm}.
\]
\[
\text{NaOMe} \quad \lambda_{\text{max}} \quad 278, 316, 416\text{ nm}.
\]
\[
\text{NaOAc/H}_2\text{BO}_3 \quad \lambda_{\text{max}} \quad 267, 297\text{sh}, 320\text{sh}, 372\text{ nm}.
\]
\[
\text{AlCl}_3 \quad \lambda_{\text{max}} \quad 260\text{sh}, 268, 303\text{sh}, 350, 424\text{ nm}.
\]

Sugars were identified as glucose and rhamnose by the usual paper chromatographic method.

Rf values of sugars in different solvent system

<table>
<thead>
<tr>
<th>Rf Value</th>
<th>Glucose</th>
<th>Rhamnose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Butanol: acetic acid: water (4:1:5)</td>
<td>- 0.12</td>
<td>0.32</td>
</tr>
<tr>
<td>2. nButanol:Ethanol:water (4:1:2.2)</td>
<td>- 0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>3. nButanol:toluene:pyridine:water (5:1:3:3)</td>
<td>- 0.24</td>
<td>0.47</td>
</tr>
<tr>
<td>4. Phenol saturated with water</td>
<td>- 0.34</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Compound H- Rutin

\[
\text{Ethanol} \quad \lambda_{\text{max}} \quad 259, 266\text{sh}, 363\text{ nm}
\]
\[
\text{Ethanol/NaOH} \quad \lambda_{\text{max}} \quad 272, 327, 415\text{ nm}.
\]
Ethanol/AlCl₃
\[ \lambda_{max} \] 275, 303 sh, 433 nm.

Ethanol + NaOAc
\[ \lambda_{max} \] 271, 325, 393 nm.

Ethanol + NaOAc/H₃BO₃
\[ \lambda_{max} \] 262, 298, 387 nm.

Rf values of rutin in different solvent system

1. Butanol:acetic acid: water (4:1:5) 0.45
2. Water 0.23
3. 15 percent acetic acid 0.51

Hydrolysis of Rutin

Rutin was hydrolysed by dissolving it in a small amount of water adding 1 percent hydrochloric acid and boiling the solution for 30 mts. The mixture was allowed to cool to room temperature and filtered. The homogeneous crystalline precipitate was washed with cold 1 percent hydrochloric acid, then with ether and filtered and dried.

Rf values of the aglycone in different solvent system

1. Conc. HCl:acetic acid: water (3:30:10) - 0.41
2. nButanol:acetic acid: water (4:1:5) 0.64
3. PhoH:water (3:1) 0.29

UV spectral data of the aglycone

\[ \text{MeOH} \]
\[ \lambda_{max} \] 255, 269sh, 301sh, 370 nm.

\[ \text{NaOAc} \]
\[ \lambda_{max} \] 257sh, 274 sh, 329, 390 nm.
The sugars were identified as glucose and rhamnose by the usual chromatographic method.

### Rf values of sugars in different solvent system

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>Rf Value</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Butanol:acetic acid: water (4:1:5)</td>
<td>0.12</td>
<td>0.32</td>
</tr>
<tr>
<td>2. nButanol:Ethanol:water (4:1:2.2)</td>
<td>0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>3. nButanol:toluene:pyridine:water (5:1:3:3)</td>
<td>0.24</td>
<td>0.47</td>
</tr>
<tr>
<td>4. Phenol saturated with water</td>
<td>0.34</td>
<td>0.60</td>
</tr>
</tbody>
</table>

### Compound 1 - Apigenin-6-8-di-C-glucoside vicenin-2

#### UV spectral data

| MeOH max                                             | 271, 333 nm. |
| NaOMe max                                            | 282, 329, 398 nm. |
| NaOAc max                                            | 280, 301sh, 387 nm. |
| NaOAc/H$_3$BO$_3$ max                                | 273, 280 sh, 320 sh, 340 sh nm. |

### Rf values of Apigenin 6,8, di-C-glucoside

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Rf Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBA</td>
<td>0.28</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.50</td>
</tr>
<tr>
<td>BAW</td>
<td>0.45</td>
</tr>
</tbody>
</table>
References


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