REFERENCES:


3-O-[α-L-RHAMNOPYRANOSYL-(1→4)-O-β-D-GLUCOPYRANOSYL] OLEAN 12-ENE-16 β-OL 28-OIC ACID FROM ANDROGRAPHIS PANICULATA (Nees.)

Saxena V.K. and Upadhyay Shruti
Department of Chemistry
Dr. H.S. Gour Vishwavidyalaya,
Sagar (M.P.) - 470 003

Abstract
The triterpenoidal saponin SU-II has been isolated from the aerial part of Andrographis paniculata (Nees.). On the basis of spectral data and chemical reactions, its structure has been established as 3-O-[α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl] 12-ene-16 β-ol 28-oic acid.

Keywords

Introduction
Andrographis paniculata (Nees.)\textsuperscript{1-3} belongs to N.O. Acanthaceae and is used as a traditional drug for treatment of liver diseases. The main biological active principle of the plant are andrographolide, androponoside and flavone which shows hepatoprotective activity and work as tonic.

The plant is distributed abundantly in the Indian northern plains and is commonly known as Kalmegh.
Result And Discussion

The aerial parts of Andrographis paniculata were extracted with ethanol and the extract was subjected to column chromatography over alumina. On elution with chloroform it gave a compound SU-II which analysed for $C_{12}H_{16}O_{13}$ m.p. 189-191°C and $M^+ = 780$. It gave all characteristic colour tests of terpene and gave positive. Liebermann-Burchard and Molisch's Test which showed that SU-II is a triterpene saponin. It also gave positive foam test.

It showed characteristic IR bands at $\nu_{\text{max}}^\text{KBr} 3377.8 \text{ cm}^{-1}$ (-OH group), 2933.9 cm$^{-1}$ (CH$_3$ str. band), 1528.1 cm$^{-1}$ (C-H str. vib.), 1233.7 cm$^{-1}$ (C=H bending of CH$_3$ gp.), 1638 cm$^{-1}$ (C=C str.) and 1066.8 cm$^{-1}$ (triterpene nucleus).

Compound SU-II on acid hydrolysis yielded an sapogenin SU-II(A) m.f. $C_{30}H_{48}O_{s}$ m.p. 280-281°C, m.f. = 472 and sugar moieties as D-glucose and L-rhamnose (R.f. 0.19 and 0.38). SU-II(A) gave all characteristic colour tests of terpene.

Presence of double bond in SU-II was indicated because solution of saponin in CCl$_4$ produced yellow colour with tetranitro methane, this was further confirmed by a band at 1405.6 cm$^{-1}$ in the IR spectrum of SU-II.

Permethylation by Kuhn procedure followed by acid hydrolysis of SU-II yielded an aglycone SU-II (A) and methylated sugars which were identified as 2, 3, 4-tri-O-methyl rhamnose (Co-Pc and Co TLC) showing the presence of D-glucose in pyranose form and also that C$_4$ of glucose was linked to C$_1$. OH group of rhamnose.

Enzymatic hydrolysis of the glycoside SU-II with Takadiastase gave prosapoagenin SU-II (Aa) and L-rhamnose (by CoPc) indicating $\alpha$-Linkage between SU-II (Aa) and L-rhamnose.
When SU-II (Aa) was further hydrolysed with almond emulsion it yielded the aglycone SU-II (A) and D-glucose. SU-II on hydrolysis with Killiani mixture liberated L-rhamnose first followed by D-glucose which suggested that L-rhamnose was attached in terminal position and D-glucose was attached to SU-II (A) aglycone directly.

The fact that the aglycone SU-II (A) and sugar (L-rhamnose and D-glucose) were present in equimolar ratio this was confirmed by Sodium metaperiodate oxidation of SU-II which consumed 2.78 moles of periodate and liberated 1.04 moles of formic acid. By this it was also confirmed that both sugars were present in pyranose form in SU-II.

The IR spectrum of SU-II (A) showed a band at 3320.2 cm\(^{-1}\) indicating the presence of -OH group(s) in it. SU-II (A) was found to form diacetyl derivative with m.f. \(C_{34}H_{52}O_{5}\) [M\(^+\)] 498 m.p. - 201-203°C. Estimation of acetyl group (19.95%) by Wiesenberger method\(^6\) as described by the Belcher and Godbert indicated the presence of two -OH group in it.

On \(Cr_2O_3/\)Pyridine oxidation, SU-II (A), yielded a di-ketone m.f. \(C_{30}H_{44}O_3\), [M\(^+\)] 452 and m.p. 215-217°C. It gave positive Zimmerman test\(^7\) for Keto group and confirmed the presence of two -OH groups, one at C-3 and other at C-16 both of secondary in nature.

A characteristic band at \(V_{\text{max}}^{KBr}\) 1630.0 and 1264.0 cm\(^{-1}\) in the IR spectrum of sapogenin SU-II (A) indicated the presence of double bond in it. Sapogenin gave yellow colour with tetranitro methane (Ruzicka's reaction)\(^1\), which indicated the presence of double bond in SU-II (A).

The sapogenin SU-II (A) showed high terminal UV absorption, characteristic of C-12 and C-13 double bond in the triterpens of oleanane series.
The $^1$H-NMR spectrum of SU-II (A) showed ethylene proton at 5.96 and 5.39 (d, $J=10.5$ Hz), which confirmed double bond at C-12 and C-13 in triterpene SU-II (A).

The IR Spectrum of SU-II (A) showed band at 2921.2 and 1452.3 cm$^{-1}$ for angular methyl groups which when estimated by Zeisels method (15.02%) confirmed the presence of seven methyl groups in it. The chemical shifts in $^1$H-NMR spectrum of SU-II (A) gave singlet at 1.05, 0.94, 0.93, 0.82, 1.16, 0.70 and 0.87 showing the presence of angular methyl groups at C-28, C-24, C-25, C-26, C-27, 29, C-30 in SU-II (A). The chemical shift recorded in $^{13}$C-NMR were 26.5, 15.2, 14.4, 18.1, 26.8, 32.8, 23.5 for C-23, C-24, C-25, C-26, C-27, C-29 and C-30 respectively.

The IR spectrum of SU-II (A) showed band at 1739.2 cm$^{-1}$ indicated the presence of $-\text{COOH}$ group, which was further confirmed by the fact that SU-II (A) gave effervescence with sodium bicarbonate solution. Sapogenine SU-II (A) on treatment with CH$_2$N$_2$/AcOH yielded mono methyl ester of SU-II (A), thereby indicating the presence of only one $-\text{COOH}$ group.

The monomethyl ester SU-II (M) analysed for molecular formula C$_{31}$H$_{56}$O$_4$, m.p. 223-224°C and [M$^+$] = 486 (FAB-MS)

Various evidences describes above on compilation indicated that the structure of SU-II was 3-O-[α-L-ramnopyranosyl-(1→4)-O-β-D-glucopyranosyl] 12-ene-16 β-ol 28-oic acid.
Experimental

Plant Material:

The aerial parts of Andrographis paniculata (Nees.) were collected locally and identified by a taxonomist.

Extraction and Isolation:

The aerial parts of Andrographis paniculata (3.0 Kg.) were air dried and defatted with petroleum ether which was further extracted with EtOH repeatedly until the extract became colourless. The concentrated mass was shaken with CHCl₃ and filtered. The residue was taken up in H₂O which extracted with n-BuOH. The n-BuOH extract was concentrated under reduced pressure when it yielded a crude compound. The crude compound was chromatographed over silica gel column and on elution with chloroform yielded SU-II (yield 0.08%) which on TLC over silica gel gave a single spot. The solvent used for TLC was Chloroform : Methanol (9 : 1) and I₂ vapours as a visualising agent.
COMPOUND SU-II:

light yellowish green powder m.p. 189-191°C, m.f. C_{42}H_{68}O_{13}, [M^+] 780, IR ν\text{max}^{\text{Kbr}} \text{cm}^{-1} 3377.8 \text{cm}^{-1} (\text{OH}), 2933.9 (\text{CH}_3 \text{ str.}), 1725.1 (\text{CH}_2-\text{CH grp.}), 1066.8 \text{cm}^{-1} (\text{triterpene nucleus}).

ACID HYDROLYSIS OF SU-II:

50mg of saponin SU-II was refluxed with 10 ml. of 7% H$_2$SO$_4$ for 7 hrs., cooled and filtered to afford sapogenin SU-II (A) which was obtained as powder of yellow colour m.p. 280-281°C.

IR: ν\text{max}^{\text{Kbr}} \text{cm}^{-1}: 3320.2 \text{cm}^{-1} (\text{OH}), 2921.2 (\text{CH}_3 \text{ str.}), 1630.0 (\text{CH}_2-\text{C}11 \text{ group}), 1390.2 \text{and} 1425.1 \text{cm}^{-1} (\text{Triterpene nucleus}) and [M^+] = 472.

$^1$H NMR:

2.88 (2H, m, H-1), 2.90 (2H, m, H-2), 3.59 (1H, m, H-3), 3.19 (1H, m, H-5), 3.54 (2H, m, H-6), 2.49 (2H, m, H-7), 1.90 (1H, m, H-9), 5.96 (1H, d, J_{11,12} = 10.5, H-12), 1.75 (1H, m, H-11), 1.50 (2H, m, H-15), 4.12 (dd, J_{15a,16} = 10.2, H-16), 1.60 (1H, m, H-18), 1.79 (2H, m, H-19), 1.43 (2H, m, H-21), 1.88(2H, m, H-22), 1.05 (3H, s, H-23), 0.94 (3H, s, H-24), 0.93 (3H, s, H-25), 0.82 (3H, s, H-26), 1.16 (3H, s, H-27), 0.70 (3H, s, H-29), 0.87 (3H, s, H-30).

$^{13}$C NMR:

37.3 (C-1), 26.1 (C-2), 89.4 (C-3), 38.4 (C-4), 54.5 (C-5), 17.2(C-6), 31.1 (C-7), 38.3 (C-8), 46.5 (C-9), 35.5 (C-10), 130.5 (C-11), 128.0 (C-12), 82.6 (C-13), 45.3 (C-14), 36.2 (C-15), 63.4 (C-16), 46.6 (C-17), 52.5(C-18), 39.2 (C-19), 32.2 (C-20), 34.3 (C-21), 25.4 (C-22), 26.5 (C-23), 15.2 (C-24), 14.4 (C-25), 18.1 (C-26), 26.8 (C-27), 32.8 (C-29), 23.5 (C-30), 75.2 (C-28).
The aqueous hydrolysate obtained after the hydrolysis of SU-II was neutralised and concentrated then chromatographed when it showed the presence of D-glucose (R₂ 0.19) and L-rhamnose (R₂ 0.38) (by PC Bu : A : W in 4 : 1 : 5).

PERMETHYLATION OF SU-II:

The saponin SU-II (30mg) was treated with methyl iodide (7 ml) and Ag₂O (40 ml) in DMF (5 ml) in a 150 ml conical flask and left for 4 days at room temperature. The contents were filtered and the residue was washed with DMF. The filtrate was concentrated under reduced pressure to get a viscous mass, which on hydrolysis with HCl gave sapogenin SU-II (A) and methylated sugars. The sapogenin SU-II (A) was separated and the aqueous hydrolysate was neutralised with BaCO₃ and BaSO₄ was filtered off and filtrate was concentrated under reduced pressure. Sugars were examined by paper chromatography using Whatmann filter paper no. 1, solvent system used was B : A : W (4 : 1 : 5) and aniline hydrogen phthalate as spraying reagent as 2, 3, 6-tri-O- methyl glucose and 2, 3, 4-tri-O-methyl-rhamnose.

PERIODATE OXIDATION OF SU-II:

The compound SU-II (25mg) was suspended in H₂O (10 ml) was mixed with NaIO₄ (250 mg) and solution was kept in dark for 48 hrs. Ethylene glycol was added to decompose excess of NaIO₄ and the solution was hydrolysed with 10% MeOH-HCl (45 min) then it was filtered and filtrate was neutralised. It did not show the presence of any mono saccharide in it.

ENZYMATIC HYDROLYSIS OF SAPONIS SU-II:

The saponin (SU-II) (40 mg) was dissolved in MeOH mixed with almond emulsion (30 ml) in a 100 ml conical flask felted with a stopper. The contents were allowed to stand at room temperature for 48 hours and then
filtered. The concentrated hydrolysate was examined on paper chromatography\textsuperscript{9} for sugar moieties using Whatmann filter paper no. 1 and B : A : W (4 : 1 : 5) as solvent system. The sugars were identified as D-glucose and L-rhamnose.

The methanolic solution of the saponin (20 mg) was mixed with an equal volume of Takadiastase solution in a conical flask. The contents were allowed to stand for 2 days at room temperature and filtered. The hydrolysate on paper chromatographic examination was found to contain L-rhamnose and D-glucose.

\textbf{Acknowledgements}

Thanks are due to the Head, Regional Sophisticated Instrumentation Centre (CDRI, Lucknow) for recording various Spectra and Head Department of Chemistry of Dr. H.S. Gour University for providing facilities.
References


To,

The Editor  
Dr. LYLE E. CRAKER  
Journal of Herbs, Spices and Medicinal Plants,  
Department of Plant and Soil Science,  
University of Massachusetts,  
Amherst, MA, 01003  
USA.

Dear Sir,

Herewith, I am submitting 4 copies of research paper entitled "Some Indegenous Plants; As Potential Hepatoprotective Agents" for favour of publication in your esteemed journal.

Hope you will please publish it.

Thanking You,

Your's faithfully

SHRUTI UPADHYAY

R/o PROF. V.K. SAXENA

Head, Department of Chemistry  
Dr. H.S. Gour University  
Sagar (M.P.) - 470 003 (India)

E-mail: albert_shalem@rediffmail.com
Amino-acid composition of some reputed hepatoprotective plants

V. K. Saxena & S. Upadhyay

Department Of Chemistry
Dr. H. S. Gour Vishwavidyalaya, Sagar (M. P.)

Key words
Amino-acids, Anthocepalus cadamin, Andrographis paniculata, Paper chromatography

Abstract
Anthocepalus cadamin\(^1\)\(^\text{3}\) and Andrographis paniculata\(^4\)\(^7\) are commonly known as Kadamba and Kalmegha.in Hindi respectively. They are reported to possess hepatoprotective activity.

Alcoholic extract of fruit of Anthocepalus cadamin has shown the presence of Threonine, Tyrosine, Methylproline and valine as amino-acid whereas an alcoholic extract of whole plant of Andrographis paniculata has shown the presence of 3, 4-dihydroxyphenyl alanine, Glutamic acid, serine, Taurine and Ethanolamine as amino acids.

Introduction
Anthocepalus cadamin\(^1\)\(^\text{1}\) (Rubiaceae) is a deciduous tree found throughout India and has a medicinal value for various orders. The bark is used as an astrigent, tonic and also in snake bite
Andrographis paniculata\(^7\) (Acanthaceae) or Kalmegh is one of the most widely used plants recommended in Charak Samhita for treatment of jaundice. It is also used for treating dysentery and for treating sluggish liver.

**Experimental**

The air-dried and powdered fruits of Anthocephalus cadambin and whole plant of Andrographis paniculata were extracted with ethanol. The extract was refluxed with 6N-HCl for 70 hours, which caused complete hydrolysis of protein into amino acids. This hydrolysed extract was subjected to ascending paper chromatography.\(^8\) Whatmann filter paper number 1 was cut into strips and spots of the hydrolysed product were applies using the fine capillary on line 1 cm. above the bottom edge.

The paper was run separately into solvent system. For Anthocephalus cadambin n-butanol : acetic acid : water (4:1:5) and for Andrographis paniculata phenol-water system (5-5.5pH) was used. Ninhydrin was used as spraying reagent for acids.

**Results and discussion**

The observation and results are tabulated below:

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>n-Butanol Acetic Acid: Water (4:1:5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spraying Reagent</td>
<td>Ninhydrin</td>
</tr>
<tr>
<td>Plant</td>
<td>Anthocephalus cadambin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S No</th>
<th>Rf Reported (^9)</th>
<th>Rf Found</th>
<th>Amino Acid Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.08</td>
<td>0.09</td>
<td>Threonin</td>
</tr>
<tr>
<td>2</td>
<td>0.15</td>
<td>0.17</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>3</td>
<td>0.31</td>
<td>0.29</td>
<td>Proline</td>
</tr>
<tr>
<td>4</td>
<td>0.51</td>
<td>0.53</td>
<td>Valine</td>
</tr>
<tr>
<td>S. No</td>
<td>Rf Reported</td>
<td>Rf Found</td>
<td>Amino Acid Identified</td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
<td>----------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>1</td>
<td>0.22</td>
<td>0.21</td>
<td>3.4-Dihydroxy phenylalanine</td>
</tr>
<tr>
<td>2</td>
<td>0.31</td>
<td>0.311</td>
<td>Glutamic acid</td>
</tr>
<tr>
<td>3</td>
<td>0.35</td>
<td>0.36</td>
<td>Serine</td>
</tr>
<tr>
<td>4</td>
<td>0.43</td>
<td>0.423</td>
<td>Taurine</td>
</tr>
<tr>
<td>5</td>
<td>0.53</td>
<td>0.542</td>
<td>Ethanol amine</td>
</tr>
</tbody>
</table>

On the basis of above table it was concluded that the plant Anthocapalus cadamin contains Threonine. Tyrosine and Valine as free amino acids whereas plant Andrographis paniculata contains 3,4-dihydroxy phenylalanine. Glutamic acid, Serine, Taurine and Ethanolamine as free amino acids.

References

PROCEEDINGS
OF THE
NINETIETH SESSION OF THE
INDIAN SCIENCE CONGRESS

BANGALORE, 2003

PART III : (Advance Abstracts)

SECTION OF CHEMICAL SCIENCES

President : Prof. S. P. Singh

CONTENTS

Sub-section                        Pages
I. Inorganic Chemistry            1
II. Organic Chemistry             46
III. Physical Chemistry           98
IV. Analytical Chemistry          137
V. Miscellaneous                  146
Dear Sir / Madam,

Your paper(s) has been accepted for Oral / Poster display during the 90th Session of the Science Congress to be held in Bangalore from January 3 to 7, 2003. Could you kindly let me know latest by Nov. 15, 2002, if you would attend the Congress and display the paper? While replying, you may kindly mention the title of your paper.

If I do not hear from you by the above date, it would be presumed that you would not be attending the Session and in that case your paper will not be included in the programme for Oral / Poster presentation.

Title of Papers –
1. ____________________________  
   S. ____________
2. ____________________________  

From
President, Section of
(Address) ____________________________

Dated ____________ 2002

Sincerely,

______________________________
117. Urs-12-Ene-16β-OI-3-O-α-D-Glucopyranoside From *Anthocephalus cadambin* Fruits

Saxena V. K. and Upadhyay S.

Department of Chemistry
Dr. H. S. Gour Vishwavidyalaya,
Sagar (M.P.)-470 003

**Key Words**: *Anthocephalus cadambin, Kadamb, Fruits, Rubiaceae,*
*Urs-12-ene-16β-OI-3-O-α-D-glucopyranoside.*

*Anthocephalus cadambin* belong to natural order Rubiaceae and is known as Kadamb. The extract of plant have been used medically for the treatment of various abnormalities.

*E.g.*: It is used as antidote in snake bite astringent to bowels and useful in blood disease. The fresh juice of the bark of plant is applied to the heads of infants when fontanella sinks.

The compound (I) \( \text{C}_{73}\text{H}_{51}\text{O}_{13} \) isolated from fruit of *Anthocephalus cadambin*. It was brown coloured which on hydrolysis with 7% \( \text{H}_{2}\text{SO}_{4} \) gave aglycone \( \text{C}_{30}\text{H}_{50}\text{O}_{2} \) and a sugar. The sugar was identified as D-glucose by PC and TLC. (Ref. 0.18).

On the basis of chemical studies and spectral data the aglycone \( \text{I}_a \) was identified as Urs-12-ene-3-α-16-β-diol, \( \text{C}_{30}\text{H}_{50}\text{O}_{2} \) and the glycoside \( \text{I} \ (\text{C}_{53}\text{H}_{51}\text{O}_{13}) \) was identified as Urs 12 ene-16-β-OI-3-O-α-D-glucopyranoside.
The Indian Science Congress Association
14, Dr. Biresh Guha Street
Kolkata 700 017.

This is to certify that Prof./Dr./Shri/Smt. [Signature] of [Institution], [City] has presented a Paper/Poster in Section of [Section Name] during the [13th] Indian Science Congress held at [City] on January 7, 2003.

His membership no is [Number].

Date: [Date]

[Signature]
Office Seal
Sectional President (Signature)
To,

Dear Dr./Mr./Ms.,

This is to inform you that your paper has been accepted for presentation at the MAPCOST sponsored 19th M.P. Young Scientist Congress to be held at Dr. H.S. Gour University, Sagar in March 2004 (fixed tentatively), subject to condition that:

1. The Abstract will be published in the name of the author who is eligible to present the paper. More than one author is not permissible, as per rules of MAPCOST, Bhopal. Please communicate the name of author (young scientist presenting the paper).

2. Proof of age has not been furnished. Please bring a copy of your H.S. Certificate.

3. Proof of experience (at least two years after PG degree in science or bachelor's degree in medical/engineering/veterinary sciences) not enclosed. Please bring proof of experience at the time of presentation.

4. Required copies (five) of paper have not been submitted. Please bring the required copies at the time of presentation.

5. Paper has not been forwarded by Supervisor/Head. Bring certificate at the time of presentation.

Prof. V.K. Saxena
Congress Co-ordinator

Prof. J.T. Rao
Chairman, Publication Committee

Prof. A.K. Banerjee
Convenor, Publication Committee
XIX M.P. Young Scientist Congress
12th - 13th April, 2004

ABSTRACTS

SOUVENIR

Sponsored by
M.P. Council of Science & Technology, Bhopal

Organised by
Dr. Hari Singh Gour University, Sagar
DOCTOR HARISINGH GOUR VISHWAVIDYALAYA, SAGAR

NATIONAL SCIENCE DAY CELEBRATIONS 2003

NEW FRONTIERS OF SCIENCE

February 28, 2003

This is in appreciation and to certify that Professor/Dr. Mr./Ms.
Shri [Name] of Chemistry dept. of Sagar
actively participated in the deliberations of the National Conference on "New Frontiers
of Science" held at Dr. Harisingh Gour University, Sagar. He/She had also presented
a paper on [Title of the paper].

Professor Santosh Kumar
Kulpati

Professor S. C. Garg
Dean, Faculty of Science
Sponsored by: M. P. Council of Science & Technology, Bhopal
Organised by: Dr. Harisingh Gour Vishwavidyalaya, Sagar

CERTIFICATE

Dr. /Mr. /Mrs. Shrutti Upadhyay of Dr. H.S. Gour University, Sagar presented his/her research paper in Chemistry discipline at the XIX M. P. Young Scientist Congress organised by Dr. Harisingh Gour Vishwavidyalaya, Sagar on 12-13 April, 2004.

Prof. V.K. Saxena
Co-ordinator
XIX MP Young Scientist Congress
Dr. H.S. Gour Vishwavidyalaya, Sagar

Prof. H.P. Garg
Principal Secretary & Director General
M. P. Council of Science & Technology, Bhopal

Prof. (Mrs.) S. Sahai
Vice-Chancellor
Dr. H.S. Gour Vishwavidyalaya, Sagar
CERTIFICATE

Mr./Ms. ___________________ has participated as a delegate in the 90th Indian Science Congress held at Jnana Bharathi Campus, Bangalore University, Bangalore during January 3rd - 7th, 2003.

Chair/ Co-Chair
Registration Committee
90th ISC, Bangalore