Phytochemicals from
Andrographis paniculata

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Andrographis paniculata is an important medicinal plant possessing
hepatoprotective and other pharmacological properties. The
detailed chemical investigation of the aerial parts of the plant
led to the isolation of one new flavone glycoside, three first time
reported compounds from the plant and a mixture of four steroids
of which three are first time reported. Besides these seven known
compounds have also been isolated.

Andrographis paniculata (Burm.f.) nees is one of the
nineteen species of Andrographis belonging to the family Acanthaceae. The plant is available abundantly in
India, Pakistan and Sri Lanka, growing in hot and shady places. It is also cultivated in certain parts of India,
East and West Indies and Mauritius.1 A. paniculata is one of the most widely used plants in Ayurvedic
formulations. At least out of forty Indian multiplant formulations, A. paniculata occurred in twenty six.2
The household medicine known as ‘alu’ in Bengal is made from its leaves and is given to children suffering
from stomach complaints and typhoid fever.3 Literature survey of the plant revealed that plant contains numer­
ous diterpenoids. The major constituent being andro­
grapholide, a bitter principle isolated from different
parts of the plant. The compound is a known hepatoprotective drug with other pharmacological properties.
Flavonoids and their glycosides are another major
group of compounds isolated from this plant.4

We have reported the antioxidant and growth inhibitory activity of plant extract and andrographolide against
Spilarctia obliqua.5 The detailed chemical investigation of the aerial parts of the plant led to the isolation of one
new flavone glycoside, three first time reported compounds from the plant and a mixture of four steroids of
which three are first time reported. Besides these seven known compounds have also been isolated.

Results and Discussion

The air-dried plant material was defatted with n-
hexane. The marc was extracted with ethanol. The
ethanolic extract thus obtained was poured in water to
get water-soluble portion and water insoluble portion.
The water insoluble part was washed with benzene
and then dissolved in methanol, which on concen­
tration gave the methanol extract. Column chromato­
graphy of n-hexane extract yielded two pure compounds and identified as pentacosane 1, hexacosyllic acid 2
and a mixture of four steroids 3-6
(ergosterol, campesterol, stigmastanol and sitosterol).
Column chromatography of methanol fraction yielded
total nine compounds 7-15. Compounds 7-11 were
identified as 14-deoxoandrographolide 7, 14-deoxy-
11,12-didehydroandrographolide 8, andrographolide
9, β-sitosterol-β-D-glucoside 10 and neoandrographo­
lide 11, respectively. Compounds 13, 14 and 15 were
identified as 5,4'-dihydroxy-7-methoxyflavone-8-O-
β-D-glucopyranoside 13, andropanoside 14 and andro­
graphiside 15, respectively. All the compounds were
characterized on the basis of spectral data and their
comparison with those reported in the literature.6-19

The compound 12 was isolated as pale yellow powder (mp 285°C, dec.) in minor quantity. It was
developed as purple coloured spot under UV light.

The positive test for sugar and flavonoid moiety suggested
that compound might be a flavonoid glycoside. The
pseudomolecular ion peak at m/z 461 [M+H]+

corresponded to the molecular formula C23H24O10

The presence of IR bands at 3496 (OH) and 1652 cm−1
(C=O) and UV absorption spectra of compound
(267.9, 333 nm) indicated the presence of flavone
skeleton.20 No sugar was released even on prolonged
boiling with acid, which confirmed the presence of C­
glycosylation. The [M-18]+ peak in mass spectrum also
supported the presence of C-glycosylation. The
[M-132]+ peak in its mass spectrum was ascribed for
aglycone, this together with a signal at δ 1.25 (brs) for
rhamnosyl methyl protons in 1H NMR, suggested that
compound 12 is a C-rhamnosyl flavone.22 The mole­
cular mass [M]+ 460 was according to flavone
C-rhamnoside bearing two hydroxyl and two methoxy
groups. The 1H NMR showed a pair of symmetric
ortho-coupled protons (2H, δ 7.97, J = 9Hz, H-2',6'
and 2H, δ 6.95, J = 9Hz, H-3',5'), characteristic for
para substituted B-ring. A singlet at δ 6.88 was
attributed to H-3. A bathochromic shift with increase
in intensity in UV spectrum with NaOMe confirmed the
presence of free OH group at C-4'. A fully substituted A-ring was ascribed by absence of any proton for ring-A. The acid stable bathochromic shift (= 17-20 nm) of both band I and band II with AlCl3 in UV spectrum and appearance of hydroxyl proton at δ 13.0 in 1H NMR confirmed the presence of free OH group at C-5 with C-6 oxygenation. This in turn fixed the position of C-rhamnosyl at C-8. The positions of remaining two methoxy groups were assigned as C-6 and C-7, which is according to the UV shift pattern with diagnostic NaOAc reagent. The anomeric proton appeared at δ 5.05 (J=7.5 Hz). Thus on the basis of above spectral features, the structure of compound was confirmed as 5, 4'-dihydroxy-6, 7-dimethoxy-flavone-8-C-α-L-rhamnoside. This compound is a new natural product.
Experimental Section

General procedure. Melting points are uncorrected. IR spectra were recorded with KBr pellets on a Perkin-Elmer 1710 FTIR spectrophotometer and are expressed in cm\(^{-1}\); UV spectra on a Perkin-Elmer Lambda Bio 20 UV spectrophotometer in methanol; and NMR spectra on a BRUKER DRX-300/DRX-200 (300MHz/200MHz) NMR spectrometer equipped with 5 mm inverse multinuclear probe head using TMS as internal standard (chemical shift were recorded in \(\delta\), ppm relative to TMS in \(^1H\) NMR whereas the residual solvent peaks were used as internal reference for \(^1H\) NMR). EIMS were obtained on a JEOL-JMSD-100 mass spectrometer at 70 eV. The electrospray mass spectra (ESMS) were recorded on a MICROMASS QUATTRO II triple quadrupole mass
spectrometer. Column chromatography was carried out on silica gel (60-120) and TLC on silica gel G (Qualigen). Spots on TLC were visualized by exposure to I_2 vapours and by spraying with 10% H_2SO_4 solution, followed by heating at 105°C for 5 minutes. Diterpenoids and their glycosides gave coloured spots, when visualized by immersing the plate in freshly prepared mixture of glacial acetic acid: conc. H_2SO_4: anisaldehyde (100:2:1).

**Plant material.** The aerial parts of *Andrographis paniculata* were collected from CIMAP farm at Lucknow and identified by the Botany and Pharmacognosy Department of our institute, where a voucher specimen has been maintained.

**Extraction and isolation.** Air dried and crushed aerial parts of *A. paniculata* (555 g) were first defatted with n-hexane (3 l/5) to obtain 20.0 g of n-hexane extract as yellowish viscous mass. The marc was then extracted with ethanol (3 l/5). The solvent was evaporated under vacuum on rotary evaporator below 50°C temperature to yield 90.0 g of ethanolic extract. The alcoholic extract thus obtained was poured in 500 mL of distilled water and subsequently filtered to get water-soluble and water insoluble portions. The water insoluble part (residue) was washed with benzene, which on concentration yielded 15.0 g of green mass. Rest was dissolved in methanol which, when evaporated under vacuum provided 25.0 g of methanol extract. Hexane extract (20 g) on column chromatography on silica-gel (350 g) yielded two pure compounds 1 and 2 along with the mixture of four steroids 3-6, where elutions were carried out in hexane-benzene changing to benzene-ethyl acetate gradient. Similarly, methanol extract (18 g) was column chromatographed on silica gel (250 g) and elutions were carried out in chloroform-methanol gradient, which yielded nine pure compounds 7-15. Each fraction was monitored by TLC and similar fractions were pooled together.

**Compound 12 (5, 4'-Dihydroxy-6, 7-dimethoxy-flavone-8-C-glycyrhamnose).** Compound was obtained as pale yellow powder (6.0 mg) in fraction eluting at MeOH : CHCl_3 : 12 : 88; mp 285°C (dec.); R_f : 0.48 (MeOH : CHCl_3 : 12 : 88); UV _λ_max (nm) : (MeOH) 267.9, 333.0; (MeOH-NaOMe) 237.5, 362.5; (MeOH-AlCl_3) 283, 352; (MeOH-AlCl_3-HCl) 284, 350; (MeOH-NaOAc) 267.8, 382.7 (sh); (MeOH-NaOAc-H_2BO_3) 267.8, 337.4; IR : 3638, 3496, 2932 (Ome), 1652, 1522 cm^{-1}; ^1H NMR (DMSO-d_6) : δ 6.88 (1H, S, H-3), 7.97 (2H, d, J= 9 Hz, H-2, 6'), 6.95 (2H, d, J= 9 Hz, H-3', 5'), 13.00 (1H, s, C-5 OH), 3.67 (6 H, brs, 2 × OCH_3), 5.05 (1H, d, J= 7.5 Hz, H-1''). 3.50-4.60 (4H, m, CH-OH sugar), 1.25 (3H, brd, H-6''); FABMS : m/z 461 [M+ H]^+ C_{25}H_{31}O_{16}, 443 [M-H_2O]^+, 329 [M-132]^+, 289 [M-132-(2 × H_2O)]^+

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**References**


