and the relative intensities (I/Io x 100) of the different peaks in the diffractograph of polymeric structures IX and XI-XIII. Compounds XI-XIII showed a similarity in the lines characteristic of structures. This indicates that these samples are isomorphous i.e. have nearly the same crystalline structure (isostructural similarities). On the other hand, the nitropolyarylate IX showed lines with very different interplanar spacings and relative intensities from the structures of XI-XIII indicating its non-isomorphism with these polymers.

The isomorphism between these nitropolyarylates XI-XIII can be attributed to the similarity between the position of the functional groups, where the dicarboxylic links are in the p-positions to each other, whereas in the polymer IX, the carbonyl links are in the m-position.

The relative intensities with two of nitropolyarylates VIII-X is represented in ***fig. 3.*** These nitropolyarylates are derived from the 5-nitroisophthaloyl chloride (I) and the different bisphenols V-VII respectively. ***Figure 3*** shows the difference in their features. This may be due to the different spatial orientation of the different groups in each bisphenol, which leads to a change in the interplanar spacings and, therefore, a change in the peak intensities, positions and numbers.

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Iridoid Glucosides from *Gentiana Kurroo* Royle

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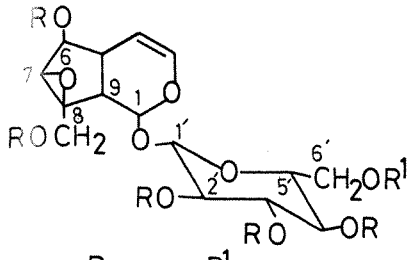
Abstract ☐ **From the ethyl acetate fraction and the aqueous residue of the ethanolic extract of *Gentiana Kurroo* Royle roots and rhizomes, six known iridoid glucosides (6-cinnamoyl- 6-0-cinnamoyl-,6-0-vanilloyl-, and 6-0-feruloylcatalpol, aucubin and catalpol) were isolated. The isolated glucosides were identified by TLC & HPLC and spectroscopic evidences (UV. IR. PMR and ^{13}C-NMR).**

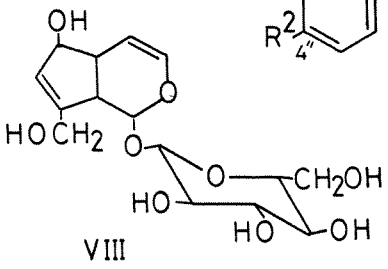
Keyphrases ☐ ***Gentianaceae, Gentiana Kurroo,* Iridoid glucosides, Catalpol esters, Catalpol, Aucubin.**

Family ***Gentianaceae*** is well known to contain iridoid and secoiridoid glycosides (1-5). ***Gentiana kurroo*** Royle is one of about 400 species representing the genous ***Gentiana*** (6-8). Its roots and rhizomes are used as bitter tonic, appetizer, anthelmintic, antiperiodic, antibilious and in treatment of fever and urinary complaints (6,9-12). Preliminary investigation of these organs revealed the presence of iridoids (13) but, no reports dealing with the chemical constituents of this plant could be

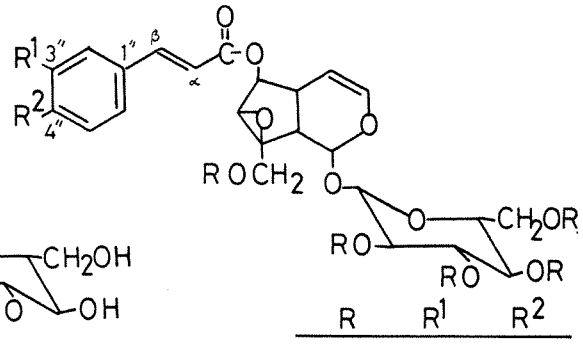
Chart :



	R	R^1
I :	H	cinnamoyl
II :	Ac	cinnamoyl
V :	H	vanilloyl
VI :	Ac	vanilloyl acetate
IX :	H	H



VIII



	R	R^1	R^2
III:	H	OH	OMe
IV:	Ac	OAc	OMe
VII :	H	H	H

traced in the literature. Therefore, it was deemed necessary to carry out the present chemical study to isolate and determine the identity of its iridoid content.

Experimental:

UV spectra were determined, for MeOH Solutions, on a Shimadzu UV-260 spectrophotometer (Japan) and IR on a Pye Unicam Sp-1000 instrument. PMR and ^{13}C-NMR spectra were determined on a varian HA-100 (XL-100) Brucker H-300 (spectrospin) Ft spectrophotometer at 200 and 25 MHz respectively with TMS as an internal standard. The chemical shift values are reported in ppm and the coupling constant are in Hz. Ms (m/z) were recorded with AEI-9 spectrometer (U.K.). Silica gel (70-230 mesh, Merck) was used for column chromatography, and silica gel GF 254 (Merck) precoated plates for TLC. Spots were visualised by UV light and by spraying with vanillin H_2SO_4 followed by heating at 120°C for 5 min. For analytical and semipreparative HPLC, a Shimadzu LC—4A HPLC instrument (Japan) was used. A shimpake ODS (C_{18}) (25 cm × 2.0 cm I.D.) column was used for semipreparative HPLC.

Plant material: The dried roots and rhizomes of authenticated ***Gentiana kurroo*** Royle were obtained from Hamdard Foundation Pakistan, Waqf, order No. 1/86 and identified by Prof. Hakeem M. Zahoorul Hassan, principal, Gov. Nizamia Tibbi College, Hyderabad, Andhara Pradesh, India.

Extraction and Isolation: About one Kg. of the powdered roots and rhizomes of ***Gentiana kurroo*** Royle was exhaustively extracted with EtOH 70% (4×5L). The hydroalcoholic extract was concentrated under reduced pressure to about 750 ml and fractionated with light petroleum 60-80°C (3 × IL), Et_2O (4 × IL) and EtOAc (6 × IL). The EtOAc fraction as well as the aqueous layer were evaporated separately to give 156.5 gm and 194.1 gm of crude glucosides, respectively.

A portion of the EtOAc fraction (15 gm) was chromatographed over silica gel (600 gm), eluted with $CHCl_3$—MeOH—H_2O mixtures (90:10:1) (IL), 80:20:2 (4L), 70:30:3 (2L) and three major fractions A—C were collected.

Fraction A: (1.8 gm) was further chromatographed over silica gel column (80 gm) using $CHCl_3$ — MeOH — H_2O (85:15:1.5) as eluting solvent to give two semipure fractions A1 and A2. Both fractions were separately purified by preparative TLC using solvent system $CHCl_3$ — MeOH — H_2O (80:20:2) to provide pure compound I (1.1 gm) and compound VII (30 mg).

Fraction B: (1.4 gm) was further fractionated by preparative TLC using $CHCl_3$ — MeOH — H_2O (75:25:2.5) as solvent system to provide two semipure fractions: B1 (100 mg) and B2 (800 mg). They were separately purified by semipreparative HPLC using MeOH — H_2O (40:60) as a mobile phase to provide 60 mg of compound III and 420 mg of compound V in a pure state.

On the other hand, a portion of the aq. residue (10 gm) was chromatographed over 400 gm of silica gel column and eluted with $CHCl_3$ — MeOH — H_2O, (80:20:2, IL), (70:30:3, 3L) and (60:40:4, 2L) and three major fractions were collected E-G.

Fraction E: (230 mg) was further purified by semiprepartive HPLC using MeOH — H_2O (10:90) as mobile phase to provide 90 mg of pure compound VIII.

Fraction F: (640 mg) was also purified in the same manner to provide 410 mg of compound IX. Isolation and identification of other iridoid glycosides from fraction C of the EtOAc fraction as well as from fraction G of the aqueous residue is in progress.

Acid hydrolysis: About 10 mg of the isolated compounds were dissolved in 5 ml of MeOH to which an equal volume of N/2 HCl was added and the mixture refluxed for 2 h on a water bath. The hydrolysate was neutralised with silver oxide and worked up in the usual way. The released sugar was identified by TLC [precoated cellulose plates. Merck, using EtOAc-pyridine-H_2O — n.BuOH — HOAc (25:20:20:50:10), and sprayed with aniline hydrogen phthalate].

6′—Cinnamoyl catalpol I: whitish amorphous substance,

Table I: **PMR Spectral Data of Compounds I-VI**

Proton(s) at C No.	Compound No. I CD_3OD	II CD_3OD	III $CDCl_3$	IV $CDCl_3$	V CD_3OD	VI $CDCl_3$
1	5.05, d (J = 9)	4.88-5.28, m	5.14, d (J = 9)	5.24, d (J = 9)	5.20, d (J = 9)	5.19, d (J = 9)
3	6.35, dd (J = 2,6)	6.30, dd (J = 2,6)	6.45, dd (J = 2,6)	6.40, dd (J = 2,6)	6.35, dd (J = 2,6)	6.36, dd (J = 2,6)
4	5.03,dd (J = 5,6)	4.88-5.28, m	5.0, dd (J = 5,6)	5.04, m	5.05, dd (J = 6,4)	5.06, m
5	2.28, m	2.55, m	2.69, m	2.69, m	2.68, m	2.69, m
6	3.65, m	4.88-5.28, m	5.10, dd (J = 7.8,1.1)	5.11, m	5.15, dd (J = 7.8,1.1)	5.12, dd (J = 7.8,1.1)
7	3.65, m	3.64 s	3.79, d (J = 1.1)	3.8, d (J = 1.1)	3.76, d (J = 1.1)	3.8, br.s
9	2.61, dd (J = 7,9)	2.55, m	2.69, m	2.69, m	2.68, m	2.69, m
10A	3.79, d (J = 13)	4.0, d (J = 13)	3.92, d (J = 13)	4.09, d (J = 13)	3.95, d (J = 13)	4.65, d (J = 13)
10B	4.17, d (J = 13)	4.84, d (J = 13)	4.15, d (J = 13)	4.86, d (J = 13)	4.25, d (J = 13)	4.95, d (J = 13)
1́	4.81, d (J = 7)	4.88-5.28, m	4.86, d (J = 7)	4.82-5.26, m	4.86, d (J = 7)	4.9-5.28, m
2́-4́	3.25-3.45, m	4.88-5.28, m	3.2-3.5, m	4.82-5.26, m	3.2-3, m	4.9-5.28, m
5́	3.25-3.45, m	3.81, m	3.2-3.5, m	3.72, m	3.2-3.5, m	3.8, m
6́	4.5, 2H, m	4.38, 2H, m	HA, 4.01,dd (J = 12,2)	4.31, dd (J = 12,2)	4.0, dd (J = 12,2)	4.33, dd (J = 12,2)
			HB, 3.73,dd (J = 12/6.3)	4.21, dd (J = 12)	3.73, dd (J = 12/6.3)	4.22, dd (J = 12/2)
H—∝	6.54, d (J = 16)	6.54, d (J = 16)	6.48, d (J = 16)	6.44, d (J = 16)	—	—
H—B	7.82, d (J = 16)	7.79, d (J = 16)	7.74, d (J = 16)	7.70, d (J = 16)	—	—
2''	7.69, d (J = 8)	7.58, d (J = 8)	7.28, d (J = 1.8)	7.20, d (J = 1.8)	7.58, d (J = 1.8)	7.60, d (J = 1.8)
3''	7.29, m	7.22-7.39, m	—	—	—	—
4''	7.29, m	7.22-7.39, m	—	—	—	—
5''	7.29, m	7.22-7.39, m	6.88, d (J = 8.2)	7.01, d (J = 8.2)	6.89, d (J = 8.2)	7.12, d (J = 8.2)
6''	7.69, d (J = 8)	7.58, d (J = 8)	7.22, dd (J = 8.2,2)	7.10, dd (J = 8.2,2)	7.11, dd (J = 8.2,2)	7.72, dd (J = 8.2,2)
$OCOCH_3$ (Aliph.)		2.03/2.05/ 2.08/2 12 ($5 \times OCOCH_3$)		2.02/2.05/ 2.07/2.13 ($5 \times OCOCH_3$)		2.01/2.05/ 2.08/2.13 ($5 \times OCOCH_3$)
$OCOCH_3$ (Arom.)				2.31 s		2.30 s
OCH_3			3.93 s	3.97 s	3.91 s	3.9 s

UVλmax: 278,222 sh,217,204 nm. IRν(KBr): 3400 (OH), 1710 (C = O, ester), 1665 (C = C, iridoid), 1635 (C = C, cinnamic acid), 1580, 1495 and 1450 cm^{-1} (aromatic ring). PMR and ^{13}C-NMR data are listed in ***Table I*** and ***II*** respectively.

6́-cinnamoyl catalpol pentaacetate II: 6—Cinnamoyl catalpol (50 mg) was acetylated with Ac_2O and pyridine at R.T. in the usual way. The product (54 mg) was obtained as amorphous substance, UVλmax:278, 222.2 sh, 217, 203 nm. IR (KBr)ν : 1750 (C = O, acetate), 1718 (C = O, ester), 1650 (C = C, iridoid), 1653 (C = C, cinnamic acid), 1580, 1495 and 1450 cm^{-1} (aromatic ring). PMR data are listed in ***Table I.*** EI—MS: m/z (rel. abundance %) : 702 (M^+, absent), 419 (9.7), 317 (0.81), 275 (0.32), 267 (0.25), 257 (4.4), 229 (0.64), 223 (0.32), 217 (0.32), 207 (1.36), 199 (0.48), 187 (0.73), 169 (2.92), 157 (0.81), 147 (5.1), 139 (1.13), 135 (0.79), 131 (100), 109 (3.9), 107 (1.8), 103 (7.2), 97 (2.6), 91 (2.6), 81 (5.2), 77 (5.2), 60 (3.9), 51 (2.6), 43 (45.5), 39 (2.6), 28 (3.9).

6—O—Feruloyl catalpol III: amorphous yellow powder : UV λ max 217.5, 235.8, 326 nm. IRν(KBr): 3351 (OH), 1702 (C = O, ester), 1665 (C = C, iridoid), 1630 (C = C, acid), 1597, 1511 and 1449 cm^{-1} (aromatic ring). PMR and ^{13}C-NMR data are listed in ***Table I*** and ***II*** respectively.

6—O—Feruloyl catalpol hexaacetate IV: 6—O—feruloyl catalpol (20 mg) was acetylated with Ac_2O at R.T. The product (23 mg) was obtained as yellowish crystalline substance (mp = 211°C), UV λ max: 217, 236, 327 nm. IRν (KBr): 1657 (C = O, acetate), 1721.4 (C = O, ester), 1650 (C = C, iridoid), 1636 (C = C, acid), 1602, 1510, and 1458

Table II: **^{13}C-NMR Spectral Data of Compounds I,III and V.**

C-Atom	6́-Cinnamoyl Catalpol I	6-O-Feruloyl Catalpol III	6-O-Vanilloyl Catalpol V
1	95.34	95.15	95.25
3	141.69	140.90	141.70
4	104.10	104.50	104.90
5	39.01	39.20	39.00
6	79.57	80.20	81.32
7	62.42	60.30	61.50
8	66.13	66.86	66.54
9	43.32	43.26	43.26
10	61.81	61.31	61.31
1'	99.72	99.78	99.77
2'	74.68	74.88	74.75
3'	77.40	78.64	78.65
4'	71.48	71.79	71.77
5'	75.77	77.76	77.72
6'	64.15	62.94	62.96
1''	135.49	127.31	122.90
2''	130.03	111.85	113.80
3''	129.27	149.58	152.90
4''	131.61	151.65	148.70
5''	129.27	116.67	115.90
6''	130.03	124.40	125.20
C-∝	146.65	147.57	——
C-ß	118.55	114.67	——
C = O	168.37	168.97	168.00
OCH_3	——	56.50	56.50

cm^{-1} (aromatic ring). PMR data are listed in ***Table I.*** EI—MS: m/z (rel. abundance %): 790 (M^+, absent), 748 (0.08), 507 (0.06), 495 (0.05), 464 (0.04), 443 (0.11), 419 (1.6), 331 (14.3), 271 (1.3), 257 (0.5), 229 (2.3), 219 (3.4), 211 (1.8), 194 (7.3), 177 (14.8), 169 (53.3), 145 (6.5), 131 (20.8), 109 (36.4), 97 (5.2), 91 (2.6), 81 (9), 71 (5.2), 57 (6.5), 43 (100) 28 (9).

6—O—Vanilloyl catalpol V: white amorphus substance, UV λ max 205, 218, 263, 292 nm. IR ν (KBr): 3367 (OH), 1703 (C = O, ester), 1653 (C = C, iridoid), 1596, 1512, 1458 cm^{-1} (aromatic ring). PMR, ^{13}C—NMR data are listed in ***Table I*** and ***II*** respectively.

6—O-Vanilloyl catalpol hexaacetate VI: 6—O—vanilloyl catalpol (40 mg) was acetylated as mentioned above. The product was obtained as white crystalline substance (mp = 180°C), UV λ max 210, 243, 290nm, IR ν (KBr): 1755 (C = O, acetate), 1720 (C = O, ester), 1659 (C = C, iridoid), 1605, 1504, 1463 cm^{-1} (aromatic ring). PMR data are listed in ***Table I.*** EI—MS: m/z (rel. abundance %): 764 (M^+, absent), 498 (0.1), 417 (0.09), 357 (0.07), 331 (13.3), 229 (2.90, 169 (44), 151 (9.3), 145 (4), 139 (2.7), 127 (7.8), 121 (2), 115 (6.7), 109 (29.3), 103 (4), 97 (4.7), 91 (4), 85 (2), 81 (6.7), 77 (2.7), 71 (4), 60 (9.3), 55 (4), 51 (2.7), 43 (100), 39 (4), 28 (9.3).

For compounds VII-IX, direct comparison of their physiochemical and spectral data (TLC, HPLC, UV, IR and NMR) with those of authentic samples of 6-O-cinnamoyl catalpol, aucubin and catalpol proved their identity respectively.

RESULTS and DISCUSSION

From the ethyl acetate fraction of the ethanolic extract of ***Gentiana kurroo*** Royle roots and rhizomes, four catalpol glucoside esters: 6′-cinnamoyl-, 6-O-feruloyl-, 6-O-vanilloyl- and 6-O-cinnamoyl catalpol were isolated through a silica gel column, preparative TLC and preparative HPLC. In the same manner, two more iridoid glucosides, aucubin and catalpol, were isolated from the aqueous layer of the same extract. Acid hydrolysis revealed the presence of glucose in all compounds under investigation. The isolated compounds were identified by spectroscopic analysis (UV, IR, MS, PMR and ^{13}C-NMR) and by direct comparison with authentic samples through chromatographic means (TLC & HPLC).

The UV and IR spectra of compound I indicated the presence of hydroxyl and ester groups and an aromatic ring (14). The PMR spectrum showed the protons of cinnamoyl moiety, glucose, and the protons of catalpol skeleton (14.15). The signals arising from the two protons at C-6′ (O 4.50) were shifted upfield by 0.7 ppm compared to those in catalpol (3.80 ppm), indicating that trans cinnamic acid is located at C-6′. Other PMR data ***(Table I)*** were identical to those of 6′—cinnamoyl catalpol (14). ^{13}C—NMR of compound I ***(Table II)*** and MS of its acetate II gave additional support for the location of cinnamic acid at C—6′. MS fragmentation showed a major fragment at m/z 419 indicating that the O-acetyl glucose oxonium ion is esterified at C-6′ with cinnamic acid and not with acetic acid (14). Fragments at m/z 267 and 207 indicated that both hydroxyl groups at C-6 and C-10 were free in compound I. All these data confirmed that compound I is 6′-cinnamoyl catalpol.

The UV and IR spectra of compound III and V indicated the presence of hydroxyl, ester groups and substituted aromatic ring. The PMR spectra show that the signals of the two protons at C-6′ and C-10 have the same chemical shift as those of catalpol (14), while those of the proton at C-6 are shifted upfield by about 1.45 ppm indicating that the hydroxyl group at C-6 is esterified by the acid moiety in both cases. Signals of C-2'', 5'' and 6'' protons at δ 7.11, 7.58, 6.11 and 7.58, 6.89, 7.11 ppm for III and V respectively (ABX-system) indicated that the aromatic ring is disubstituted at C-3'' and C-4''. In addition signals of one OCH_3 group at 4.03 ppm and signals of one aromatic acetate at 2.30 ppm proved that the aromatic ring is substituted by a hydroxyl at C-4'' and by a OCH3 group at C-3''. In case of compound III, it was clear that the acid moiety has signals at 6.48 & 7.74 ppm dd, J = 16 Hz characteristic of trans α and ß protons, but compound V lacks these signals. These data led to the conclusion that compound III is catalpol esterified by ferulic acid at C-6 and compound V is esterified by vanillic acid at C-6. ^{13}C-NMR of compounds III and V and MS of their acetates IV and VI respectively gave an additional support for the location of ferulic and vanillic acids at C-6 of the catalpol skeleton in compounds III and V respectively. MS fragmentation of their acetates IV and VI showed a peak at m/z 331 corresponding to the tetraacetylglucose oxonium ion (16). Spectral data of compound III were identical with those

of 6-O-feruloyl catalpol (17.18) and that of compound V were identical to 6-O-vanilloyl catalpol (18).

Spectral data of compound VII-IX were identical to those of 6-O-cinnamoyl catalpol, aucubin, and catalpol (14,15) respectively. further confirmation of the identity was obtained by direct chromatographic (TLC, HPLC) and spectroscopic (UV,IR) comparison with authentic samples.

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Analysis of Pharmaceutical Formulations Containing Ampicillin and Cloxacillin Using Second-Derivative Spectroscopy on a Diode-Array Spectrophotometer.

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Abstract ☐ **Two-component mixture of ampicillin and cloxacillin with considerable overlapping spectra was assayed by a second-derivative spectrophotometric method using a diode-array spectrophotometer. The second-derivative spectra of sample solutions in distilled water were scanned between 260 and 300 nm. Derivative measurements were carried out at 269 nm (zero-crossing), and at 288 nm (peak-to-zero) for ampicillin and cloxacillin, respectively. The procedure does not require any separation step or chemical reagents and allows for a single-unit assay for normal dosage used. Beer's law holds up to 14 mg% for both penicillins. The method was successfully applied to commercial preparations containing a binary mixture of both penicillins.**

Keyphrases ☐ **Ampicillin, cloxacillin, derivative spectrophotometry, simultaneous determination, diode-array spectrophotometer.**

Derivative ultraviolet-visible spectroscopy is a technique that offers a versatile solution to a number of analytical problems (1-3), such as the resolution of multicomponent systems (4), removal of sample turbidity and matrix background (5,6) determination of a drug in presence of its synthetic precursors or degradation products (7,8) and enhancement of spectral details (9). The application of derivative spectrophotometry to multicomponent mixtures reveals its usefulness in this field. Derivative spectrophotometry has been employed for the determination of the following drugs alone or in mixture; acepifylline and phenobarbitone, phenylbutazone and