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Research Paper Organic Chemistry

Analgesic activity of Anacardium occidentale

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ABSTRACT

The fresh flowers of Anacardium occidentale Linn., cashewnut, is examined to contain the flavonoids quercetin and isoquercitrin. Modern physical methods like UV, NMR and chemical reactions, PC and hydrolytic studies were used to ascertain the structure. The analgesic property of the isolated glycoside has been found to contain ample analgesic activity as it is compared with a standard.

Keywords : Anacardium occidentale, quercetin, isoquercitrin, albino rats.

Introduction

Anacardium occidentale Linn., Popularly Known as Mundiri Kottai, in Tamil, is a small spreading, ever green tree, sometimes reaching a height of 12 m, native to tropical America, and naturalized in the warmer parts of India especially near the sea[1].

The acrid oil stated above as cardole is often applied to floors or wooden rafters of house to prevent the attack of white ants, and most effectually keeps them away. A transparent gum is obtained from the trunk of the tree, useful as a good varnish, and making a fair substitute for gum-Arabic. It should be collected while sap is rising. It is particularly useful when the depredations of insects require to be guarded against. For this purpose it is used in South America by the bookbinders, who wash their books with a solution of it in order to keep away moths and ants.[2]

Experimental

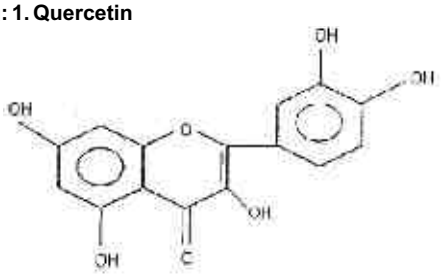
Extraction and Fractionation

Fresh flowers (1 kg) of A.occidentale collected from in and around Jayankondam, Perambalur District, during May-June were extracted with 85% methanol (5 X 500 ml) under reflux. The alcoholic extract was concentrated in vacuo and the aqueous concentrate was successively fractionated with petroleum ether (60-80C) (4 X 250 ml) peroxide - free Et_2O (3 X 250 ml) and EtOAc (4 X 250).The petrol fraction did not yield any crystalline solid and could not be studied further.

Et_2O Fraction (flavonol: quercetin)

The Et_2O fraction was concentrated in vacuo and left in and ice-chest for a week. A yellow solid that separated was filtered and studied. On crystallization from MeOH, pale yellow needles were obtained [Gl, m.p. 313-15C, yield 0.028]. It was readily soluble in organic solvents and sparingly in hot water. It gave a red color with Mg-HCl, Olive- green color with alc. Fe^{3+}, golden-yellow color with NH_3 and NaOH, yellow solution with a pale green fluorescence with conc. H_2SO_4 and appeared yellow under UV and UV/NH_3. It reduced ammonical $AgNO_3$ in the cold and Fehling's solution on heating. It answered the Horhammer-Hansel [3], Wilson's boric acid[4] and Gibb's[5] tests. It gave a pentaacetate, m.p. 200-01C and a pentabenzoate m.p 188-90C. It had ^{MeOH} 255, 269 sh, 370: NaOMe 262 sh, 322, 420 (dec.) + $AlCl_3$ 267, 303, 458; + ($AlCl_3$ - HCl) 267,303,351, 428; + NaOAc 275,328,390

Fig: 1. Quercetin

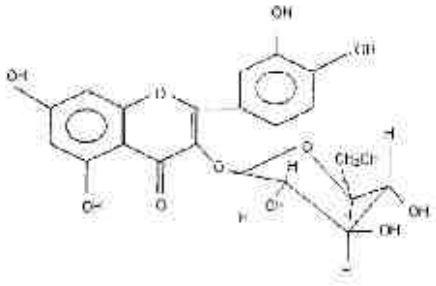


and + ($NaOAc-H_3BO_3$) 262,303 sh, 386 nm and had R_f values as depicted in Table I-1. The ^{1}H-NMR of the flavonol is appended. It was identified as quercetin and the identity as confirmed by CO- mixed-PC and m.m.p with an authentic sample of quercetin from Physalis minima [6].

Ethyl acetate fraction: (flavonol glycoside-isoquercitrin)

The EtOAc fraction was concentrated in vacuo and left in an ice-chest for 2 days. A yellow solid that separated was filtered and studied. It was recrystallised from MeOH when it afforded yellow crystals, m.p 229-30C (yield 0.1%). It was freely soluble in EtOAc and MeOH and sparingly in water. It gave an olive- green colour with alc. $FeCl_3$ deep pink colour with Mg - HCl, yellow colour with NaOH and appeared deep purple under UV that turned yellow on exposure to NH_3. It did not answer the Horhammer-Hansel test but responded to the Wilson's boric acid. Gibb's and Molich's tests. The pigment was

Fig: 2. Isoquercitrin



homogeneous on TLC and had R_f as indicated in Table I-2 and had λ^{MeOH} nm 257,269 sh,299 sh,362; + NaOMe,272,327,409;+AICI3 275, 303 sh; 333, 430; + ($AICl_3$ HCI) 274,303 sh, 353, 401 ;+ NAOAC 271,320 sh, 372, and, +($NaOAc-H_3BO_3$) 265, 300 sh, 372. The ^{1}H-and ^{13}C-NMR of the glycoside are appended (Figs). It was identified as isoquercitrin by comparing it with an authentic sample isolated from **G.ulmifolia.**

Table-I : R_f (X 100) Values Of The Constituents Of The Flowers Of Anacardium Occidentale (Whatman No.1, Ascending, 30 ± 2C)

Compound	Developing solvents									
	a	b	c	d	e	f	g	h	i	j
Glycoside From A.occidentale	3	7	22	34	59	60	57	6	67	68
Isoquercitrin (authentic)	3	6	23	35	60	59	55	66	66	68
Aglycone of The above glycoside	-	-	4	18	41	84	47	48	71	87

TABLE II : R_f (X 100) values of the sugar from the glucoside from *a.occidentale*

Compound	Developing solvents			
	e	f	g	i
Sugar from the Hydrolysate of EtOAc fraction	77	09	39	90
Glucose (authentic)	77	09	39	90

Hydrolysis of the glycoside

To a solution of the glycoside (0.lg, 0.2 m mole) in hot aq. methanol (10ml, 50 %) an equal volume of H_2SO_4 (10%) was added and the mixture refluxed at 100C for 2hr. The aq. Hydrolysate was worked up in the usual way as mentioned under ***A.occidentale.***

Identification of aglycone (flavonol: quercetin)

The yellow pigment from the above hydrolysate was identified as quercetin as described under Et_2O fraction.

Identification of Sugar (glucose)

The aq.solution from the above hydrolysate was neutralised with $BaCO_3$ and filtered. The concentrated filtrate on chromatographic examination (PC) gave R_f values corresponding to those of glucose. The running properties of the glycoside were in favour of a monoside. The identity of the sugar was also confirmed by direct comparison with an authentic sample of glucose.

Table-III : ^{13}C-NMR And Signal Assignment For The Aglycone From The Flowers Of Anacardium Occidentale

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Quercetin (from literature) (δ ppm)	146.9	135.8	175.9	160.8	98.3	164.0	93.5	156.2	103.1
Aglycone Isolated	146.838	135.709	175.840	160.727	98.295	164.426	93.427	156.211	102.990

Compound	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
Quercetin (from literature) 254 (δ ppm)	122.1	115.2	145.1	147.1	115.7	120.1
Aglycone Isolated	122.035	115.154	145.110	147.755	115.683	120.026

Table-IV : ^{13}C-NMR And Signal Assignment For The Glycodide From The Flowers Of Anacardium Occidentale

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Glycoside Isolated	156.421	135.516	177.645	161.417	98.873	164.288	93.728	156.421	104.187

Compound	C-1	C-2	C-3	C-4	C-5	C-6
Glycoside	121.384	115.423	144.976	148.631	116.212	121.801
Compound	C-1″	C-2″	C-3″	C-4″	C5″	C-6″
Glycoside	101.078	74.288	76.112	69.747	77.316	60.797

Results And Discussion

The flowers of **A.occidentale** have been found to contain quercetin and its isoquercitrin. The UV spectrum of the flavonol aglycone obtained from the ether fraction λ_{max}^{MeOH} at 370 nm (band I) and 255 nm (band II) indicating a flavonol skeleton. Its NaOMe spectrum degenerated with time. Flavonols Possessing free-OH groups at the 3-3'- and 4' positions are known to be unstable in NaOMe. It could therefore be inferred that there are free- OH groups at C-3, C-3'and C-4' in the compound. A shift of + 58nm on the addition of $AICl_3$ - HCI is indicative of the presence of a free OH at C-5 in the A-ring.

A comparison of $AICl_3$ and $AICl_3$ HCI spectra revealed an additional bathochromic shift of 30nm in the case of $AICl_3$ spectrum (without acid), which again points to the presence of catechol type of B-ring. The presence of a free-OH at C-7 is evident from the bathochromic shift of 20nm, in band II on the addition of NaOAc. The presence of a catechol type of B-ring is also evident from the bathochromic shift of 16nm noticed in band I on addition of H_3BO_3.

In the ^{1}H-NMR-7 spectrum (400 MHZ. DMSO-d_6; TMS) Fig-2 of the aglycone the hydroxyl proton at C-5 shows up at 12.4799 ppm as a distinct singlet.

The Signal of 9.469 ppm corresponds to - OH proton C-3. The doublet at 8.04 and 8.02 ppm accounts for the hydroxyl protons at C-3' and C-4' the C-5' protons appears as a doublet at 6.91 ppm (j=8.5 HZ). The Signals due to the protons at C-2 and C-6 overlap at 7.53 ppm ring protons at C-6 and C-8 could be located respectively at 6.17 and 6.40 ppm. Based on this observation the aglycome has been unambiguously characterized as quercetin.

The fresh flowers of ***A. occidentale*** have been found to contain quercetin and isoquercitrin.

The UV Spectrum of the aglycone exhibited 2 major peaks at 370 nm (band I) and 255 nm (band II) to reveal a flavonol skeleton. Decomposition was observed on the addition NaOMe to the aglycone. Since flavonols which have free hydroxyl groups at the 3,3' and 4' positions are unstable in NaOMe and the absorption peaks in NaOMe spectrum degenerate in a few minutes, it was inferred that there was free-OH group at C-3,C-3' and C-4' in the compound. A shift of + 58 nm on the addition of $AICl_3$- HCI showed the presence of a free 5-OH in the A-ring.

Comparing the $AICl_3$ spectra, + 30 nm shift was observed in the case of $AICl_3$ without acid, which also revealed a B-ring O-dihydroxyl group. The presence of a free-OH at C-7 was ascertained by a shift of + 19 nm (band II) on the addition of NaOAc. The catechol type of dihydroxyl group in B-ring was further evidenced by the bathochromic shift 18 nm on the addition of H_3BO_3.

The UV spectrum of the glycoside showed two major absorption peaks at 362 nm (band I) and 257nm (band II) showing a flavonol skeleton. A bathochromic shift of 47 nm in band I observed in its NaOMe spectrum indicated the presence of a free 4'OH group. The $AICl_3$-HCI spectra of the glycoside as well as its aglycone showed 3 absorption peaks and a shoulder indicating a free 5-OH group in both. The glycoside as well as its aglycone did not exhibit any intense UV fluorescence ascertaining the presence of a free hydroxyl in $AICl_3$ - HCI spectra was yet another evidence for the same.

The presence of a C-7-OH group is evident from a shift of +14 nm in the case of the glycoside and +19 nm in the case of the aglycone on addition of NaOAc. The presence of an O-dihydroxyl Group in the B-ring could be inferred from a shift of +10 nm noticed in the glycoside and +18 nm noticed in case of the aglycone on the addition of H_3BO_3. In the AICI3 spectrum, an absorption peak was noticed at 430 nm (band I) which on addition of HCI reduced by 29 nm. This another evidence for the presence of a catechol type di-OH group in the B-ring.

In the ^{1}H-NMR spectrum (290 MHZ, DMSO-d6, TMS) of the glycoside, the protons at C-6 and C-9 appear, as doublets at 6.2 and 6.4 ppm respectively. The 5-OH proton resonates at 12.7 ppm as a distinct singlet. The C-5' proton appear as a doublet at 6.8 ppm .The H-1" signal of the flavonol 3-O-glycoside is found at 5.9 ppm. The remaining glucosyl Protons appear in the range 3.4 to 3.5 ppm.

Supporting evidence for the structure of the glycoside was provided by the analysis of ^{13}C-NMR (67.89 MHZ, DMSO-d_6 TMS) data a complete assignment is given (Table). Due to glycosylation at 3-position, C-2 and C-4 carbons absorb at 101 ppm. The rest of the carbons of the sugar unit appear between 77.3 and 60.9 ppm.

Based on this the glycoside has been characterized as isoquercitrin (quercetin 3-O-glycoside).

Analgesic activity examination

Methods and materials

The Glassman's method was employed for the assessment of analgesic activity. Albino Swiss were divided into groups of six animals each (20-25 grams). They were fasted initially for 16 hours. Group I served as control (Normal saline 2 ml / Kg). Group 2 & 3 were administered ethyl acetate extract of ***Anacardium occidentale*** (100 mg / Kg & 200 mg / kg) by oral route respectively. Group 4 served as standard (Pentazocine 5 mg / kg) by intraperitoneal route. The time of reaction to pain stimulus of the mice placed on the hot plate heated at 55 ± 0.5° C was recorded at 30 min., 60 min., 120 and 180 min., after administration of test drug. The increase in reaction time against control was calculated.

Fig: 3 : Drug Administration

Fig : 4 : Mouse Kept at 55.5° C

Fig 5 : Tolerance Limit Observation

TABLE-5 : Analgesic Effect Of Anacardium Occidentale By Hot Plate Method

Treatment	Reaction time in sec.				
Time in Minutes	0	30	60	120	180
Control	4.4± 0.93	4.6± 0.82	4.9± 0.83	4.1± 0.52	4.4± 0.38
Anacardium occidentale (100 mg / kg)	4.2± 0.71	4.9± 0.35	5.7± 0.24	6.3± 0.15	6.5± 0.37
Anacardium occidentale (200 mg / kg)	4.4± 0.37	6.3± 0.35	8.6± 0.36	9.6± 0.47*	10.0± 0.31*
Pentazocine (5mg / kg)	4.3± 0.46	6.8± 0.87	8.4± 0.67	10.0± 0.43*	10.0± 0.46*

Data are expressed mean ± S.E,n = 6
*P <0.01 vs Control by students 't' test

Results And Discussion

The yellow pigment isolated from the flowers of ***A.occidentale*** was examined for its analgesic property by Hot plate Method.

At a lower concentration of 100 mg/Kg body weight of the mice, two different concentrations were applied. The reaction time was observed at 0, 30th, 60$^{th.}$ 120th and 180th minute. A control without drug showed approximately 4.9 seconds. While 100 mg/kg body weight was administered the reaction time increases with the above mentioned intervals. It goes to the maximum level of 6.5 ± 0.37 seconds. As the concentration of the drug is increased to 200 mg / kg body weight substrantial analgesic property is noticed. The value gets elevated from 4.4 ± 0.37 at the starting reaches 10.0± 0.31 at 180th minute. Thus the drug shows the maximum analgesic property while it is compared with a standard drug of pentazocine of 5 mg/kg body weight.

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