STUDIES ON THE CONSTITUENTS OF FORMOSAN *SALVIA PLEBEIA* R. BR. (I)

ON THE FLAVONOID COMPONENTS OF *SALVIA PLEBEIA* R. BR.

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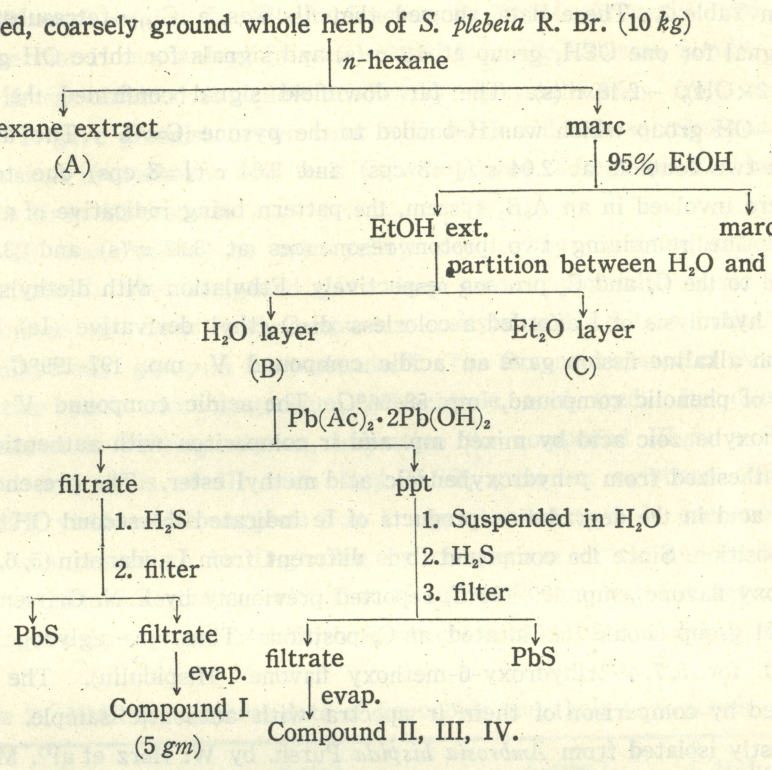
Salvia plebeia R. Br. is an annual herb of Labiatae and is widely distributed in the central area of Taiwan. It has been used as folk-medicine for the treatment of hepatitis and tumors.[1] From the flavonoid enriched fraction (B) of the alcoholic extracts of this plant, four kinds of flavonoid compound were isolated. Two of these were confirmed as 5, 4′-dihydroxy-6-methoxy-7-glucosyloxy flavone (I). (homoplantaginin, hispidulin-7-glucoside) and 5, 7, 4′-trihydroxy-6-methoxy flavone (II) (hispidulin) on the basis of their spectral, chemical evidences and comparison with authentic samples. The other two were assigned to be 5, 3′, 4′-trihydroxy-6-methoxy-7-glucosyloxy flavone (III) (nepitrin, eupafolin-7-glucoside) and 5,7,3′,4′-tetrahydroxy-6-methoxy flavone (IV) (eupafolin, nepetin) on the basis of chemical and spectral properties. The flavones, II (hispidulin) and IV (eupafolin), have been shown to have cytotoxic activity against human carcinoma of the nasopharynx carried in cell culture (KB).[13]

Salvia plebeia R. Br. is an annual herb of Labiatae and is widely distributed in the central area of Taiwan. It has been used as folk-medicine for the treatment of hepatitis and tumors[1]. Many species of Salvia genus have been extensively investigated[2] for their chemical constituents. However, there are no scientific reports on the *Salvia plebeia* R. Br.. In the course of our systematic study on Formosan antitumor plants[3], we have examined the chemical constituents of *Salvia plebeia* R. Br. in view that various species of Salvia show antitumor activity as reported by J. L. Hartwell[4]. In this paper, we like to report the isolation and characterization of the flavonoidal components of this plant. The preliminary fractionation of the alcoholic extract is summarized in scheme I.

The flavonoid enriched fraction (B) afforded four kinds of flavonoid compound (I, II, III, IV).

Compound (I) was crystallized from MeOH as yellow needles, mp. 254-256°C, $[\alpha]_D^{30.5} = -73°$ (c=1, pyridine), UV. λ_{max}^{EtOH}: 336 $m\mu$ (log ε 4.50), 277 $m\mu$ (log ε 4.36). This gave a greenish brown color with ferric chloride solution, and the flavonoid character was shown by the usual reduction test with magnesium-hydrochloric acid (red)[5]. Elemental analysis supported the molecular formula $C_{22}H_{22}O_{11}\cdot 2H_2O$. It formed hexaacetate (Ia),



Scheme 1

$C_{34}H_{34}O_{17}$ (M^+ 714), mp. 232-234°C. Its dilute acid hydrolysate reduced the Fehling solution, indicating that the compound (I) is a flavonoid glycoside. Hydrolysis of I with 10% H_2SO_4 afforded an aglycone (Ib) in 59.7% yield, which suggests that it contains one molecule of sugar. The sugar portion was treated by the usual process and D-glucose was detected by thin layer chromatography. Its osazone as orange yellow needles, mp. 199-203°C, showed no melting point depression (mixed mp. 199-205°C) on admixture with glucosazone (mp. 206-208°C), and their ir spectra also found to be identical. The aglycone (Ib), mp. 292-294°C, $[\alpha]_D^{27} = \pm 0$ (c=1.06, pyridine), gave positive magnesium-hydrochloric acid test (orange red) and ferric chloride test (greenish brown). The elemental analysis and a mass spectrum supported the molecula formular $C_{16}H_{12}O_6$ (M^+ 300). The ir (KBr) spectrum showed the absorption bands at 3400 cm^{-1} (broad, OH) and 1650 cm^{-1} (conjugated C=O). The UV. absorption spectra, λ_{max}^{EtOH} :337 $m\mu$ (log ε=4.49), 276 $m\mu$ (log ε=4.42); $\lambda_{max}^{EtOH+AlCl_3}$: 357 $m\mu$, 304 $m\mu$, indicated that it is a flavone derivative with C_4'-substitution and not a flavonol.[6] Further, the bathochromic shift of both band I (20 $m\mu$) and band II (28 $m\mu$) on addition of aluminum chloride to an ethanolic solution of Ib indicated the presence of C_5—OH group.[6] The NMR spectral data for aglycone (Ib), its triacetate (Ic), mp. 169-171°C, $C_{22}H_{19}O_9$ (M^+ 426) and trimethyl ether (Id), mp. 142-143°C, $C_{19}H_{19}O_6$ (M^+ 342), are

given in Table 2. These data showed that Ib was a $C_{5674'}$-tetrasubstituted flavone, with signal for one OCH_3 group at 6.2 τ (s) and signals for three OH groups at 0.2 τ (broad, 2×OH), −2.48 τ (s). The far downfield signal confirmed the presence of a free C_5—OH group which was H-bonded to the pyrone C=O.[7] The aromatic region exhibits two doublets at 2.04 τ (J=8 cps) and 3.04 τ (J=8 cps) due to four protons that were involved in an A_2B_2 system, the pattern being indicative of a $C_{4'}$-substituted B-ring. The remaining two proton resonances at 3.22 τ (s) and 3.40 τ (s), were assigned to the C_8 and C_3 protons respectively. Ethylation with diethylsulfate followed by acid hydrolysis of I afforded a colorless di-O-ethyl derivative (Ie), mp. 204-205°*C*, which on alkaline fission gave an acidic compound V, mp. 197-199°*C* and a minute amount of phenolic compound, mp. 58-60°*C*. The acidic compound V was confirmed as *p*-ethoxybenzoic acid by mixed mp. and ir comparison with authentic sample which was synthesized from *p*-hydroxybenzoic acid methyl ester. The presence of *p*-ethoxybenzoic acid in the degradation products of Ie indicated the second OH group locating at $C_{4'}$-position. Since the compound Ib is different from Landanetin (5, 6, 4′-trihydroxy-7-methoxy flavone), mp. 190-192°*C*, reported previously by E. N. Gritsenko et al[8], the third OH group should be located at C_7-position. Thus, the aglycone (Ib) could be assigned for 5, 7, 4′-trihydroxy-6-methoxy flavone (Hispidulin). The identity was confirmed by comparison of their ir spectra with authentic sample. Hispidulin has been firstly isolated from *Ambrosia hispida* Pursh. by W. Herz et al[9]. Methylation of I with diazomethane gave its di-O-methyl ether (Ig), mp. 172-174°*C*, ir (KBr): 3400 cm^{-1} (OH), 1640 cm^{-1} (conjugate C=O), which was identified as di-O-methyl homoplantaginin by mixed mp. and ir comparison with that of authentic sample. This fact indicated the compound I to have a 5, 4′-dihydroxy-6-methoxy-7-glucosyloxy flavone structure (Homoplantaginin, hispidulin-7-glucoside). By comparison of the ir spectrum with that of authentic chart confirmed the identity. Homoplantaginin has been firstly

Table 1. UV. spectra data for flavonoids

Compound	$\lambda_{max}^{EtOH} m\mu$ (log ε)	$\lambda_{max}^{EtOH+AlCl_3} m\mu$	$\lambda_{max}^{EtOH+AcONa} m\mu$
I	277 (4.36)	287 (inf.)	277
Homoplantaginin	336 (4.50)	302	336
		356	
Ib (II)	276 (4.42)	296 (inf.)	278
Hispidulin	337 (4.49)	304	338
		357	
IIIa (IV)	257 (4.37)	279	277
Nepetin (Eupafolin)	274 (4.37)	300 (inf.)	363
	350 (4.48)	400	

isolated from *Plantago asiatica* L. (Plantaginaceae) by Dr. M. Aritomi[10] and this is the first instance that Homoplantaginin was isolated from the Labiatae family.

Compound II was obtained as yellow needles by recrystallization from methanol, mp. 289-291°C. It gave greenish brown color with ferric chloride solution and orange red with magnesium-hydrochloric acid test. It was proved to be hispidulin by direct comparison with Ib in all respects.

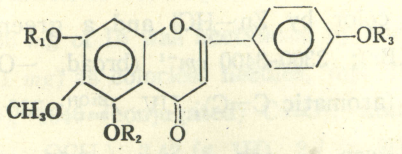
Compound III was isolated as pale yellow needles by recrystallization from MeOH, mp. 265-266°C. The compound gave deep green color with ferric chloride solution and brown red color with ammonium molybdate-HAc test indicating the presence of o-dihydroxyl group in the molecule[14]. The flavonoid character was showed by the reduction test with magnesium-hydrochloric acid (red). Its dilute acid hydrolysate reduced the Fehling solution, indicative the compound III is a flavonoid glycoside. Acid hydrolysis of III with 10% H_2SO_4 gave an aglycone (IIIa), mp. 269-271°C, in 62% yield, which suggests that a sugar bonded to it is one mole. The sugar portion was proved to be D-glucose by comparison of tlc and the ir (KBr), mixed mp. of its osazone derivative with that of authentic samples. The aglycone IIIa mp. 269-271°C, gave deep green color with ferric chloride solution and red color with

Table 2. NMR (τ) spectral data of flavonoids and their derivatives

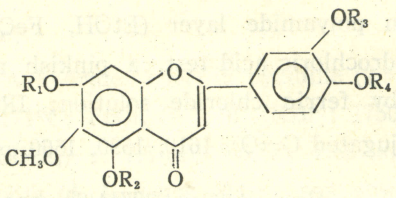
Compound	A-ring	B-ring				C-ring	OCH_3	Hydroxyl or Acetate
	C-8H	C-2′H	C-3′H	C-5′H	C-6′H	C-3H		
Ib (DMSO) Hispidulin	3.22s	2.04d	3.04d (A_2B_2; J=8 cps)	3.04d	2.04d	3.40s	6.22s	C-5OH −2.48 C-7OH, C-4′OH 0.2
Ic ($CDCl_3$) Hispidulin triacetate	2.70s	2.14d J=8 cps	2.70s	2.70s	2.14d J=8 cps	3.40s	6.12s	C-5OAc 7.5 C-7OAc 7.6 C-4′OAc 7.65
Id ($CDCl_3$) Hispidulin trimethyl ether	3.20s	2.16d	3.00d (A_2B_2; J=8 cps)	3.00d	2.16d	3.40s	6.00s 6.09s 6.12s	
IIIa (DMSO) Eupafolin (Nepetin)	3.41s	2.67m		3.10d J=8 cps	2.67m	3.48s	6.32s	C-5OH −3.2 C-7OH 0.81 C-3′OH 0.39 C-4′OH −0.40
IIIb ($CDCl_3$) Eupafolin tetraacetate	2.60d J=8 cps	2.35m		2.60d J=8 cps	2.35m	3.40s	6.10s	C-5OAc 7.50 C-7OAc, C-3′OAc, C-4′OAc 7.60, 7.80

magnesium-hydrochloric acid. The ir spectrum showed bands at 3400 cm^{-1} (broad, OH) and 1655 cm^{-1} (conjugated C=O). The UV absorption data are given in Table 1. The λ_{max}^{EtOH} of band I at 350 $m\mu$ indicated a flavone rather than a flavonol and the appearance of band II as two peaks at 274 $m\mu$ and 253 $m\mu$ suggested that B-ring was 3′4′-disubstituted[6]. Upon the addition of aluminum chloride to ethanolic solution of the flavone, there was a large bathochromic shift of 50 $m\mu$ in band I, and band II appeared as a single peak at 279 $m\mu$, indicated the presence of free C_5—OH group[6]. A bathochromic shift to 277 $m\mu$ of the low wavelength band on the addition of sodium acetate indicated the presence of free C_7—OH group[6]. The NMR spectral data for compound IIIa and its acetate (IIIb) are given in Table 2. These data showed that the aglycone IIIa was penta-substituted, with signal for one OCH_3 group at 6.32 τ and signals for four OH groups at 0.81 τ, 0.39 τ, −0.40 τ and −3.2 τ. The far-downfield signal confirmed the presence of C_5—OH group. The aromatic region of the NMR showed a one-proton doublet centered at 3.1 τ (J=8 cps) and multiplet signals centered at 2.67 τ corresponding to two protons, the pattern being indicative of a 3′4′-disubstituted B-ring. The remaining two singlets at 3.48 τ and 3.41 τ could be assigned to the C_3 and C_8 protons. The NMR spectrum of acetate (IIIb) exhibited three singlet signals at 7.5 τ, 7.6 τ and 7.80 τ coressponding to four $COCH_3$ groups, confirmed the presence of four OH groups in IIIa, and one singlet at 6.1 τ for one OCH_3 group. In addition to the above spectral properties, both III and IIIa gave positive test with ammonium molybdate-acetic acid (brown-red), indicative that two hydroxyl groups are located at 3′4′ position. Moreover, IIIa was different from pedalitin[11], which is an aglycone of pedaliin from sesame leaves and revised independently the structure as 5, 6, 3′, 4′-tetrahydroxy-7-methoxy flavone by Krishnaswamv N. R. et al[12] and S. M. Kupchan et al[13], as comparison of their described melting point in literature. Hence the structure of IIIa could be assigned for 5, 7, 3′, 4′-tetrahydroxy 6-methoxy flavone, (nepetin, eupafolin) and III for 5, 3′, 4′-trihydroxy-6-methoxy 7-glucosyloxy flavone (nepitrin, eupafolin-7-glucoside), according to the physical and chemical evidences as described above. The glucoside III has been isolated from *Nepeta hindostana*[12] and *Rosmarinus officinalis* L.[15]

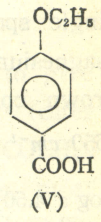
Compound IV was isolated as yellow needles, mp. 269-271°C. The compound gave positive ferric chloride test (deep green) and magnesium-HCl test (red). It was identified as eupafolin (IIIa) by direct comparison of polyamide layer chromatography, ir spectra and mixed melting point. Eupafolin (IIIa, IV) also found in *Eupatorium cuneifolium*[13]. The flavones, hispidulin (Ib, II) and eupafolin (IIIa, IV), have been identified as the cytotoxic compounds of Eupatorium species by S. M. Kupchan et al[13].



(I): R_1=Glucose
R_2=R_3=H
(Ia): R_1=tetraacetyl glucose
R_2=R_3=$-COCH_3$
(Ib)=(II): R_1=R_2=R_3=H
(Ic): R_1=R_2=R_3=$-COCH_3$
(Id): R_1=R_2=R_3=CH_3
(Ie): R_1=H R_2=R_3=C_2H_5
(Ig): R_1=Glucose
R_2=R_3=CH_3



(III): R_1=Glucose
R_2=R_3=R_4=H
(IIIa): R_1=R_2=R_3=R_4=H
(IIIb): R_1=R_2=R_3=R_4=$-COCH_3$
(IIIc): R_1=R_2=R_3=R_4=C_2H_5
(IV)=(IIIa)



(V)

EXPERIMENTAL

Extraction and isolation of flavonoids

Salvia plebeia R. Br. was collected in the central area of Taiwan in May, 1970 by Professor W. S. Kan. The air dried, coarsely ground whole plant material (10 *kg*) was steeped with *n*-hexane (A) and extracted ten times with 95% ethanol. Evaporation of the alcoholic extract under reduced pressure left a dark green viscous residue (1 *kg*). The residue was treated with water and partitioned between water (B) and ether (C). The water layer (B), which is the flavonoid enriched fraction, was treated with saturated lead subacetate solution, giving non-precipitable and precipitable fractions. After removal of Pb, the non-precipitable fraction was concentrated under reduced pressure to give compound (I) (5 *gm*). This was crystallized from methanol as yellow needles, mp. 254-256°*C*. The precipitable fraction was suspended in water and was saturated with hydrogen sulfide to remove Pb. Evaporation of the filtrate under reduced pressure gave a brown thick syrup (100 *gm*), from which was obtained brownish yellow crystals (crude compound III, mp. 253-256°*C*, 6.5 *gm*) on addition of alcohol and letting stand for one week. Solvent was removed from the filtrate under reduced pressure. A portion of the residue (25 *gm*) was chromatographed on a column (3×42 *cm*) of silicic acid (200 mesh, Wako), eluted with $CHCl_3$, 1% MeOH—$CHCl_3$ and finally with 10% MeOH—$CHCl_3$. Fractions were combined on the basis of polyamide layer chromatography (EtOH, $FeCl_3$ solution). Fractions 6-8 (1% MeOH—$CHCl_3$) yielded compound II as yellow needles (500 *mg*), mp. 289-291°*C*. Fractions 12-18 (1% MeOH—$CHCl_3$) yielded compound IV as yellow needles (410 *mg*), mp. 269-271°*C*. The fractions eluted by 10% MeOH—$CHCl_3$ gave compound III (80 *mg*) as fine yellow needles, mp. 265-266°*C*.

Compound I (Homoplantaginin)

Yellow needles from MeOH, mp. 254-256°*C*, $[\alpha]_D^{30.5}=-73°$ (c=1, pyridine), showed

single spot on polyamide layer (EtOH, $FeCl_3$). It gave orange red color with magnesium-hydrochloric acid test, a pinkish red color by Zn—HCl and a greenish brown color by ferric chloride solution; IR (KBr): 3300-3400 cm^{-1} (broad, —OH), 1650 cm^{-1} (conjugated C=O), 1610, 1590, 1560 cm^{-1} (aromatic C=C); UV. λ_{max}^{EtOH}: 336 $m\mu$ (log ε 4.50), 277 $m\mu$ (log ε 4.36); $\lambda_{max}^{EtOH+AlCl_3}$: 356 $m\mu$, 302 $m\mu$, 287 $m\mu$ (inf.); $\lambda_{max}^{EtOH+AcONa}$: 336 $m\mu$, 277 $m\mu$. Anal. calcd. for $C_{22}H_{22}O_{11}\cdot 2H_2O$: C, 53.01; H, 5.22. Found: C, 53.06; H, 5.19. The ir (KBr) spectrum superimposes with the authentic Homoplantaginin spectrum by Dr. M. Aritomi.

Acetylation of I, homoplantaginin hexaacetate (Ia)

A solution of I (141 *mg*) and acetic anhydride (2.5 *ml*) in pyridine (2 *ml*) was allowed to stand at room temperature for two days. The reaction mixture was poured into 25 *ml* of ice water and stirred for 50 minutes. White precipitate collected and recrystallized from methanol furnished a quantitative yield of Ia as colorless rods, mp. 232-234°*C*, $FeCl_3$ (—). It showed a molecular ion peak at m/e 714 and other prominent peaks at m/e 672 (M-42), 384, and 342. Anal. calcd. for $C_{34}H_{34}O_{17}$: C, 57.28; H, 4.76; Found: C, 56.90; H, 4.94.

Acid hydrolysis of I, formation of hispidulin (Ib) and glucose

1.025 *gm* of I was refluxed with 10% H_2SO_4 (30 *ml*) and EtOH (30 *ml*) on water bath for 12 hrs. After removal of EtOH, the separated aglycone (Ib) was collected, washed with water and dried at 100°*C* for 1 hr. The yield of Ib (613 *mg*) was 59.7% to I. The aqueous solution free from the aglycone was neutralized with $BaCO_3$ and condensed under reduced pressure. This concentrated solution, which gave positive reaction to Fehling solution and showed a single spot on tlc layer (SiO_2, *n*-propanol: EtOAc: H_2O=7:2:1, anisaldehyde-H_2SO_4 solution) with the same Rf value as that of D-glucose, was warmed on boiling water with phenylhydrazine HCl (970 *mg*) and sodium acetate (1.2 *gm*) for 20 minutes to give osazone as orange yellow needles, mp. 199-203°*C*. It showed no depression on admixture with glucosazone which was prepared from D-glucose in the same manner. Their ir spectra are also identical.

Ib (hispidulin): mp. 292-294°*C* from MeOH; Mg—HCl (red), $FeCl_3$ (greenish brown); ir (KBr): 3400 cm^{-1} (broad, —OH), 1650 cm^{-1} (conjugated C=O), 1600 cm^{-1} (aromatic C=C); UV. λ_{max}^{EtOH}: 337 $m\mu$ (log ε 4.49), 276 $m\mu$ (log ε 4.42); $\lambda_{max}^{EtOH+AlCl_3}$: 357 $m\mu$, 304 $m\mu$, 296 $m\mu$ (inf.), $\lambda_{max}^{EtOH+AcONa}$: 338 $m\mu$, 278 $m\mu$; mass spectrum, m/e: 300 (M^+), 285 (M-15), 282, 257 (M-15-28); nmr (DMSO) τ: 6.22 (s, C_6—OCH_3), 3.40 (s, C_3—H), 3.22 (s, C_8—H), 3.04 (d) and 2.04 (d) (A_2B_2, J=8 cps, 4H for $C_{3'5'}$ and $C_{2'6'}$), 0.2 (broad, 2×OH), −2.48 (s, C_5—OH). Anal. calcd. for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03. Found: C, 63.76; H, 3.92. Its ir spectrum is superimposable with that of authentic hispidulin.

Acetylation of Ib, hispidulin triacetate (Ic)

86 *mg* of Ib was acetylated by acetic anhydride (3 *ml*) in pyridine (2.5 *ml*) to give Ic (71 *mg*) as colorless needles, mp. 169-171°*C*; $FeCl_3$ (−); ir (KBr): 1750 cm^{-1} (ester C=O), 1640 (conjugated) C=O); nmr ($CDCl_3$) τ: 7.5 (s), 7.6 (s), 7.65 (s) ($3\times COCH_3$), 6.12 ($1\times OCH_3$), 3.42 (s, 1H), 2.7 (s, 3H), 2.14 (d, J=8 cps, 2H); mass spectrum, m/e: 426 (M^+), 384 (M-42), 342 (384−42), 327 (342−15). Anal. calcd. for $C_{22}H_{18}O_9$: C, 61.99; H, 4.26. Found: C, 62.43, H, 4.08.

Methylation of Ib, hispidulin trimethyl ether (Id)

Methylation was carried out by adding KOH solution (2 *gm*/8 *ml* H_2O) dropwise to a mixture of Ib (70 *mg*), MeOH (10 *ml*) and dimethyl sulfate (4 *ml*) at room temperature. After removal of methanol, the precipitate was collected and purified over neutral Al_2O_3 colum through the elution with $CHCl_3$ to furnish Id (50 *mg*) as colorless crystals, mp. 142-143°*C*. The nmr ($CDCl_3$) spectrum showed signals at 6.0 τ (s), 6.09 τ (s), 6.12 τ (s) (12H, $4\times OCH_3$), 3.4 τ (s, 1H), 3.2 τ (s, 1H), 3.0 τ (d, J=8 cps, 2H), 2.16 τ (d, J=8 cps, 2H). The mass spectrum, m/e 342 (M^+), 327 (M-15). Anal. calcd. for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30. Found: C, 67.12; H, 5.60.

Ethylation and hydrolysis of I, hispidulin di-O-ethyl ether (Ie)

A mixture of I (617.5 *mg*), anhydrous potassium carbonate (15 *gm*), acetone (50 *ml*) and diethylsulfate (10 *ml*) was refluxed on water bath for 12 hrs. After filtration, the clear reaction mixture was distilled in vacuo. The residue was refluxed with 40 *ml* of 10% H_2SO_4 and 10 *ml* EtOH on water bath for another 14 hrs. The reaction mixture was evaporated and extracted with ether. The total ether extract was washed with water, dried over $MgSO_4$ and evaporated to afford a light green solid. Chromatographic elution with $CHCl_3$ on SiO_2 column gave colorless plates of Ie (250 *mg*), mp. 204-206°*C*. The ir (KBr) spectrum showed absorption bands at 3400 cm^{-1} (OH), 1640 cm^{-1} (conjugated C=O), 1600 cm^{-1}, and 1560 cm^{-1} (aromatic C=C).

Alkaline degradation of Ie

A mixture of Ie (200 *mg*), 50% KOH (20 *ml*) and EtOH (10 *ml*) was refluxed under N_2 for 40 hrs. After removal of EtOH, the reaction mixture was acidified with 30% H_2SO_4 and extracted with ether (4×50 *ml*). The ether layer was washed with 5% $NaHCO_3$ (4×20 *ml*), dried over $MgSO_4$ and distilled. The residue was purified by recrystallization from $CHCl_3$-*n*-hexane (1:1) to afford a light pinkish needles, mp. 58-60°*C*; ir (KBr): 3500 cm^{-1} (OH), 3200 cm^{-1} (broad, bonded OH), 1600 cm^{-1} (aromatic C=C). The sodium bicarbonate extract was acidified with diluted HCl and extracted with ether (4×50 *ml*). This was dried over $MgSO_4$ and evaporated to afford a white solid. Two recrystallizations from EtOH gave a colorless plates (35 *mg*), mp. 197-199°*C* (sintered at 185°*C*), which was identified as *p*-ethoxy benzoic acid by mixed mp. and ir comparison with that of authentic sample.

Synthesis of p-ethoxy benzoic acid

Methyl paraben (0.5 *gm*) was refluxed in a mixture of 20% KOH (10 *ml*), MeOH (10 *ml*) and diethyl sulfate (3 *ml*) for 2 hrs. The reaction mixture, after removal of MeOH, was diluted with water (10 *ml*) and warmed on boiling water bath for 30 minutes, acidified with concentrated HCl and cooled. The white precipitate was collected and recrystallized from EtOH to give colorless scales (200 *mg*), mp. 197-199°*C*.

Methylation of I with diazomethane, di-O-methyl homoplantaginin (Ig)

An excess Et_2O solution of CH_2N_2 was charged to I at room temperature for 4 days. The solvent was removed and the residue was recrystallized from methanol to give light yellow needles, mp. 172-174°*C*. It showed no depression on admixture with that of authentic di-O-methyl homoplantaginin (Ig) and the ir spectra were identical.

Compound II (hispidulin, Ib)

Yellow needles from MeOH, mp. 289-291°*C*, gave positive tests with Mg—HCl (orange red) and $FeCl_3$ (greenish brown). It was identical to the aglycone (Ib) of I in all respects (IR, TLC and mixed mp.).

Compound III, Nepitrin

Pale yellow needles from MeOH, mp. 265-266°*C*. It gave red color with Mg-HCl, deep green color with $FeCl_3$ (on polyamide layer) and brown red color on ammonium molybdate-acetic acid test. The acid hydrolysate reduced Fehing solution. IR (KBr): 3200-3400 cm^{-1} (broad, bonded OH), 1650 cm^{-1} (conjugated C=O), 1600 cm^{-1}, 1570 cm^{-1} (aromatic C=C).

Acid hydrolysis of III, nepetin (eupafolin) (IIIa) and glucose

54.5 *mg* of III was refluxed with 10% H_2SO_4 (6 *ml*) and EtOH (10 *ml*) for 7 *hrs*. The aglycone (IIIa) was collected, washed with water and dried at 100°*C* for one hour. The yield of IIIa (33.8 *mg*) was 62% to III. The aqueous solution free from the aglycone (IIIa) was neutralized with $BaCO_3$ and evaporated in vacuo. The concentrated solution gave a single spot on tlc (SiO_2, *n*-propanol: EtOAc: H_2O=7:2:1, anisaldehyde-H_2SO_4 solution) with the same R_f value as that of authentic *D*-glucose. The osazone is orange yellow needles, mp. 199-202°*C*, which shows identical melting point and ir spectrum to an authentic sample of glucosazone.

IIIa (nepetin, eupafolin): mp. 269-271° from MeOH. This gave positive tests with Mg-HCl (red), $FeCl_3$ (deep green), ammonium molybdate-HAc (brown red) and Zn-HCl (violet); UV. λ_{max}^{EtOH}: 350 *mμ* (log ε=4.48), 274 *mμ* (log ε=4.37), 257 *mμ* (log ε=4.37); $\lambda_{max}^{EtOH+AlCl_3}$: 400 *mμ*, 300 *mμ* (inf.), 279 *mμ*; $\lambda_{max}^{EtOH+AcONa}$: 363 *mμ*, 277 *mμ*; $\lambda_{max}^{EtOH+EtONa}$: 415 *mμ*, 278 *mμ*; ir (KBr): 3400 cm^{-1} (broad, OH), 1655 cm^{-1} (conjugated C=O); nmr (DMSO):

6.32τ (s, C_6-OCH_3), 3.41τ (s, C_8-H), 3.48τ (s, C_3-H), 3.1τ (d, J=8 cps, $C_{5'}$-H), 2.67τ (m, 2H, $C_{2'6'}$-H), 0.81τ, 0.39τ, −0.40τ (s, 3H, $C_{7\ 3'4'}$-OH) and −3.2τ (s, C_5-OH).

Acetylation of IIIa, eupafolin tetraacetate (IIIb)

Acetylation of IIIa (128 *mg*) by acetic anhydride-pyridine method gave IIIb as colorless needles (116 *mg*), mp. 179°*C* (lit.[13] 181-3°*C*). NMR ($CDCl_3$): 7.8τ, 7.6τ, 7.5τ (s, 4×OAc), 6.1τ (s, 1×OCH_3), 3.40τ (s, 1H), 2.6τ (d, J=8 cps, 2H), 2.35 (m, 2H).

Ethylation of IIIa, nepetin tetraethyl ether (IIIc)

A mixture of IIIa (160 *mg*), diethyl sulfate (2 *ml*), anhydrous K_2CO_3 (2 *g*) and acetone (15 *ml*) was refluxed on water bath for 7 hours. The mixture was filtered and the solvent was removed by distillation in vacuo. The residue was treated with water and filtered. The precipitate was recrystallized from MeOH as white needles, mp. 142-144°*C* (lit. 145-147°*C*[12], 124-126°*C*[13]).

Compound IV

Yellow needles from MeOH, mp. 269-271°*C*, deep green to $FeCl_3$, red color with Mg-HCl, violet color with Zn-HCl and brown red to ammonium molybdate-HAc. It showed no mp. depression on admixture with IIIa and the ir spectra as wall as tlc were also identical.

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