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Carlos Chaverri

Centro de Investigaciones en
Productos Naturales
(CIPRONA) and Escuela de
Química, Universidad de Costa
Rica. San José, Montes de Oca,
San Pedro, 11501-2060 COSTA
RICA.

José F. Ciccío

Centro de Investigaciones en
Productos Naturales
(CIPRONA) and Escuela de
Química, Universidad de Costa
Rica. San José, Montes de Oca,
San Pedro, 11501-2060 COSTA
RICA.

Leaf and flower essential oil compositions of *Gliricidia sepium* (Fabaceae) from Costa Rica

Carlos Chaverri, José F. Ciccío

Abstract

Gliricidia is a small genus of flowering plants in the Fabaceae family. *Gliricidia sepium* is a multipurpose tree native to Mesoamerica and possibly northern South America. The chemical composition of the hydrodistilled essential oils of *G. sepium*, growing wild in the Central Pacific coast of Costa Rica, was analyzed by capillary gas chromatography-flame ionization detector (GC-FID) and capillary gas chromatography-mass spectrometry (GC-MS) using the retention indices on DB-5 type capillary column. A total of 96 and 109 compounds were identified in the leaf and flower oils, respectively, corresponding to 87.9% and 89.2% of the total amount of the oils. The leaf oil consisted mainly of aliphatics (54.9%) and terpenoids (28.1%). The major compounds from the leaf oil were pentadecanal (18.7%), (*Z*)-phytol (7.8%), methyl linolenate (6.0%) and nonanal (5.1%). The flower oil consisted mainly of aliphatics (58.9%) and terpenoids (25.8%). The major components of the flower oil were hexadecanoic acid (19.7%), myrtenol (7.7%) and (*E*)-nerolidol (5.9%).

Keywords: *Gliricidia sepium*, Fabaceae, essential oil composition, hexadecanoic acid, pentadecanal, Costa Rica.

1. Introduction

The Fabaceae (Leguminosae) is a large family of flowering plants with about 727 genera and over 19300 species distributed throughout the world [1]. Around the world, the family has great economic value because some species are important agricultural plants utilized for food (*Phaseolus* L. spp., beans, *Glycine max* (L.) Merr., soya, *Pisum sativum* L., peas, *Arachis hypogaea* L., peanut, etc.) Also, some species are farmed as a source of timber for construction and furniture.

Gliricidia Kunth comprises a small number of taxa. It is constituted of low-branching flowering trees distributed mainly in tropical America. The most prominent species in this plant genus is *G. sepium* known for presenting large numbers of pink flowers which attract carpenter bees, mainly *Xylocopa fimbriata* [2] and by its dark hardwood with good potential for making pulp and paper [3].

Gliricidia sepium (Jacq.) Kunth ex Walp, is commonly known as “Madero negro” in Costa Rica. It is a fast growing tree which is used in the tropics for firewood, lumber, fodder and soil improvement. The tree is used as living fence posts and to provide shade in coffee and cacao plantations. It is native to Central America ranging from southern Mexico to northern South America where it occurs in a number of ecological zones and at altitudes ranging from sea level to 1200 m [4, 5]. The prevalence of natural stands in the tropical dry forest of the Pacific slope might indicate that this environment represents the true natural habitat of the species in Costa Rica [6].

Gliricidia sepium is a small to medium-size deciduous tree (usually about 2-15 m tall). The plant presents alternate, pinnately compound leaves, of 15 to 35 cm long, the leaflets are elliptical or lanceolate, 3 to 6 cm long and 1.5 to 3 cm wide. There are 6-24 opposed leaflet pairs and a terminal leaflet. They are dull green and pale grayish beneath, sometimes tinted bronze or purple. The inflorescences are racemes (showy clusters) and have 5 to 15 cm long, borne at the base of leaves. The flowers are pink, pink-lavender with splash of yellow or white with a conspicuous red-brown calyx [4, 7, 8]. Seed pod (legume) is dark brown, oblong, flat, from 10 to 12 cm long and 1.5 to 2 cm wide.

The leaf decoction of *G. sepium* is used in several countries as an expectorant and as a febrifuge and cold remedy [7]. Also, the leaves are used in baths for malaria, and poultice on

Correspondence:

José F. Ciccío

Centro de Investigaciones en
Productos Naturales
(CIPRONA) and Escuela de
Química, Universidad de Costa
Rica. San José, Montes de Oca,
San Pedro, 11501-2060 COSTA
RICA.

skin erysipelas, bruises and sores [7]. In Guatemala and Costa Rica, the bark decoction is used against protozoal diseases and for the treatment of impetigo and other skin diseases [7]. Leaves, roots and seeds are toxic to mice and giant pocket gophers ("taltuzas") [9]. Some traditional uses and biological activities of several extracts of *G. sepium* were listed and reviewed recently by Lim [10].

The phytochemistry of *G. sepium* has been extensively investigated. This plant contains mainly flavonoids and allied phenolics [11-16], lignans [17], other aromatics [18-20] and triterpenic saponins [21, 22]. Previously, the composition of the essential oils from leaves, flowers [23] and bark [24] of *G. sepium* cultivated in Kerala, India, was published.

The aim of this work was to examine the chemical composition of the oils obtained from the leaves and flowers of *G. sepium* growing wild in the Central Pacific coast of Costa Rica.

2. Materials and methods

2.1 Plant Materials

Leaves and flowers of *G. sepium* (Fabaceae), growing wild in Costa Rica, were collected during the flowering period in March-April, 2009, near Judas de Chomes (10° 06' 27.58" N, 84° 54' 46.94" W, 38 m elevation) in the Pacific coast, Province Puntarenas, Costa Rica. A voucher specimen was deposited in the Herbarium of the University of Costa Rica (USJ 93891).

2.2 Isolation of the essential oils

The fresh leaflets and flowers were subjected to hydrodistillation at atmospheric pressure, for 3 h using a modified Clevenger-type apparatus. The distilled oils were collected and dried over anhydrous sodium sulfate, filtered and stored at 0-10 °C in the dark, for further analysis. The yield of the pale yellowish oil from the leaflets was 0.012 % (v/w) and from the flowers was 0.005% (v/w).

2.3 Gas chromatography (GC-FID)

The oils of *Gliricidia sepium* were analyzed by GC-FID (gas chromatography with flame ionization detector) using a Shimadzu GC-2014 gas chromatograph. The data were obtained on a poly (5% phenyl-95% methylsiloxane) fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 µm), (MDN-5S, Supelco), with a LabSolutions, Shimadzu GC Solution, Chromatography Data System, software version 2.3. Operating conditions were: carrier gas N₂, flow 1.0 mL/min; oven temperature program: 60-280 °C at 3 °C/min, 280 °C (2 min); sample injection port temperature 250 °C; detector temperature 280 °C; split 1:60.

2.4 Gas chromatography-mass spectrometry (GC-MS)

The analyses by gas chromatography coupled to mass selective detector were performed using a Shimadzu GC-17A gas chromatograph coupled with a GCMS-QP5000 apparatus and CLASS 5000 software with Wiley 139 and NIST computer databases. The data were obtained on a poly (5% phenyl-95% methylsiloxane) fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 µm), (MDN-5S). Operating conditions were: carrier gas He, flow 1.0 mL/min; oven temperature program: 60-280 °C at 3 °C/min; sample injection port temperature 250 °C; detector temperature 260 °C;

ionization voltage: 70 eV; ionization current 60 µA; scanning speed 0.5 s over 38-400 amu range; split 1:70.

2.5 Identification of the constituents

The oil components were identified using the retention indices (RI) on DB-5 type column [25] and by comparison of their mass spectra with those published in the literature [26] or those of the author's database. To obtain the retention indices for each peak, 0.1 µL of *n*-alkane mixture (Sigma, USA, standard mixture C₈-C₃₂, R 8769) was injected under the same experimental conditions reported above. Integration of the total chromatogram (GC-FID), expressed as area percent, has been used to obtain quantitative compositional data.

3. Results and Discussion

The composition of the hydrodistilled essential oils from leaves and flowers of *Gliricidia sepium* are presented in Table 1. The components are listed in order of elution on a MDN-5S column. Table 1 gives the relative percentages of single components, their experimental retention indices (RI) with reference to a homologous series of linear alkanes (C₈-C₃₂) and, for purposes of comparison, literature values (Lit. RI) are given.

The chemical analysis of the leaf oil showed that the major classes of constituents were aliphatics (54.9%) and terpenoids (28.1%) (See Table 2, where the percentages of the various classes of constituents of the oils are indicated). Among the 58 aliphatic compounds identified, the major constituents were aldehydes (ca. 30%). Pentadecanal (18.7%) and nonanal (5.1%) were the main ones. Of the twenty-six terpenoids identified, none was found in high concentration. The main ones were pinocarvone (1.5%), myrtenol (1.3%), β-cyclocitral (1.1%), α-pinene (1.0%) and β-caryophyllene (1.0%). These results differ markedly from those reported for the chemical composition of *G. sepium* leaf oil obtained from plants growing at Kerala, southwestern India on the Malabar Coast [23]. Kaniampady *et al.* reported that the leaf essential oil was characterized by large amounts of propylene glycol (25.1%), (the authors claimed that it was the first report of propylene glycol obtained from a natural source), coumarin (18.2%), (*Z*)-3-hexen-1-ol (17.7%) and β-farnesene (14.2%) [23]. The data of the oil samples from Costa Rican leaves do not appear to confirm the presence of that main constituent and afforded only minute amounts of (*Z*)-3-hexen-1-ol (0.2%) and coumarin (0.8%).

The flower oil consists mainly of aliphatics (58.9%) with 67 compounds identified (Table 2) and terpenoids (25.8%) with 35 compounds identified. The major components of the flower oil (Table 1) were hexadecanoic acid (19.7%), myrtenol (7.7%), (*E*)-nerolidol (5.9%), linoleic acid (3.4%), and heptacosane (3.1%). The flower oil of India [23] contains coumarin (43.1%), hydroquinone (21.6%) and myrtenol (12.7%). Of the 26 compounds reported in the Indian sample [23] only five compounds were present in Costa Rican samples. The flower oil from Costa Rica lacks coumarin and hydroquinone, the major compounds from Indian oil sample. Also, the chemical composition of the flower oil differs markedly from that of India. The flower oil composition was characterized by unbranched homologous series of hydrocarbons (C₁₃-C₂₉), alcohols (C₆-C₁₂), aldehydes (C₆-C₁₈), ketones, fatty acids, esters and many unsaturated aliphatic

compounds. Some of these compounds are probably biosynthesized from fatty acids. Among the group of the monoterpenoids, several compounds were identified with a

typical pinane carbon skeleton: α -pinene, *trans*-pinocarveol, pinocarvone (1.8%), pinocampheol, myrtenol (7.7%), *cis*- and *trans*-myrtenols and myrtenyl acetate.

Table 1: Chemical and percentage composition of the essential oils from leaves and flowers of *Gliricidia sepium*.

Compound ^a	RI ^b	Lit. RI ^c	Class	Leaf %	Flower %	Identification method ^d
Methylbenzene	788	773 ^[27]	B	0.1	1.8	1,2
Hexanal	801	801	A	0.2	0.1	1,2
5- <i>tert</i> -Butyl-1,3-cyclopentadiene	839	839	Misc	0.1	nd	1,2
(<i>E</i>)-3-hexen-1-ol	849	844	A	0.2	0.7	1,2
(<i>Z</i>)-3-Hexen-1-ol	850	850	A	0.2	nd	1,2
(<i>E</i>)-2-Hexen-1-ol	855	854	A	nd	0.4	1,2
(<i>Z</i>)-2-Hexen-1-ol	859	859	A	0.2	nd	1,2
Hexan-1-ol	862	863	A	0.3	1.6	1,2
Heptan-2-one	889	889	A	0.1	nd	1,2
(<i>Z</i>)-4-Heptenal	890	893	A	t	nd	1,2
Heptanal	901	901	A	t	0.1	1,2
2-Ethyl pyrazine	912	912	Misc	t	nd	1,2
2-Methyl-4-pentenoic acid	925	929	A	0.5	nd	1,2
α -Pinene	934	932	M	1.0	0.1	1,2,3
Benzaldehyde	950	952	B	0.2	0.1	1,2,3
Heptan-1-ol	961	959	A	0.1	0.1	1,2
Isoamyl propanoate	965	960	A	0.1	nd	1,2
1-Octen-3-one	972	972	A	0.1	nd	1,2
1-Octen-3-ol	977	974	A	1.7	1.0	1,2
Octan-3-one	982	979	A	0.1	0.1	1,2
2-Pentylfuran	988	984	Misc	0.2	0.3	1,2
Octan-3-ol	988	988	A	0.2	0.1	1,2
Octanal	998	998	A	0.2	nd	1,2
(<i>E</i>)-3-Hexenyl acetate	1002	1001	A	nd	t	1,2
3-Methyl-1,2-cyclopentanedione	1019	1017	A	0.6	0.2	1,2
<i>p</i> -Cymene	1021	1020	M	nd	0.1	1,2
Limonene	1024	1024	M	nd	t	1,2,3
(<i>E</i>)-3-Octen-2-one	1030	1030	A	0.7	nd	1,2
2,2,6-Trimethylcyclohexanone	1033	1036 ^[28]	A	0.2	nd	1,2
Salicylaldehyde	1039	1039	B	0.3	0.1	1,2
(<i>E</i>)-2-Octenal	1042	1049	A	nd	t	1,2
(<i>E</i>)-2-Octen-1-ol	1065	1060	A	0.3	0.4	1,2
Octan-1-ol	1069	1063	A	0.3	0.3	1,2
Linalool	1098	1095	M	1.4	0.6	1,2,3
Nonanal	1100	1100	A	5.1	0.8	1,2
Heptyl acetate	1112	1112	A	nd	0.1	1,2
<i>exo</i> -Fenchol	1119	1118	M	0.3	nd	1,2
1-Terpineol	1136	1130	M	nd	0.6	1,2
<i>trans</i> -Pinocarveol	1139	1135	M	0.1	0.1	1,2
<i>neo</i> -Isopulegol	1146	1144	M	nd	0.2	1,2
(<i>Z</i>)-3-Nonen-1-ol	1153	1152	A	1.4	2.4	1,2
iso-Isopulegol	1155	1155	M	nd	2.0	1,2
2-Hydroxyacetophenone	1156	1155	B	2.4	nd	1,2
(<i>E</i>)-2-Nonenal	1157	1157	A	nd	0.3	1,2
Pinocarvone	1162	1160	M	1.5	1.8	1,2
(2 <i>E</i> ,6 <i>Z</i>)-Nona-2,6-dien-1-ol	1165	1160 ^[29]	A	0.5	nd	1,2
(<i>E</i>)-2-Nonenol	1167	1163	A	0.9	2.6	1,2
Pinocampheol	1166	1166	M	nd	t	1,2
(<i>Z</i>)-6-Nonenol	1170	1164	A	nd	t	1,2
Nonan-1-ol	1168	1165	A	1.0	0.2	1,2
Menthol	1167	1167	M	nd	0.1	1,2
Terpinen-4-ol	1174	1174	M	nd	0.1	1,2,3
Thuj-3-en-10-al	1181	1181	M	nd	0.1	1,2
<i>trans-p</i> -Mentha-1(7),8-dien-2-ol	1188	1187	M	0.3	0.1	1,2
Methylsalicylate	1192	1190	B	0.3	0.2	1,2
Myrtenol	1196	1194	M	1.3	7.7	1,2
Safranal	1199	1197	M	0.6	nd	1,2
Decanal	1206	1201	A	0.8	0.3	1,2,3

<i>trans</i> -Pulegol	1213	1213	M	0.2	nd	1,2
β -Cyclocitral	1219	1217	M	1.1	nd	1,2
Nerol	1224	1227	M	0.7	0.3	1,2
(<i>Z</i>)-3-Hexenyl-2-methylbutanoate	1227	1229	A	0.3	nd	1,2
Geraniol	1250	1249	M	0.7	0.7	1,2
<i>cis</i> -Myrstanol	1254	1250	M	nd	0.3	1,2
Carvenone	1258	1255	M	nd	0.1	1,2
<i>trans</i> -Myrstanol	1259	1258	M	0.8	0.4	1,2
Geranial	1268	1264	M	0.2	nd	1,2
Decanol	1270	1266	A	nd	0.4	1,2
Undecan-2-one	1293	1293	A	nd	t	1,2
(2 <i>Z</i> ,4 <i>Z</i>)-Decadienal	1296	1292	A	nd	0.3	1,2
Tridecane	1300	1300	A	0.4	0.1	1,2,3
Undecanal	1306	1305	A	nd	0.5	1,2
<i>p</i> -Vinyl-guaiacol	1308	1309	B	0.8	nd	1,2
(2 <i>E</i> ,4 <i>E</i>)-Decadienal	1315	1315	A	0.3	1.0	1,2
(<i>E</i>)-3-Hexenyl tiglate	1317	1315	A	0.2	nd	1,2
Myrtenyl acetate	1323	1324	M	0.5	0.1	1,2
1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene (=α-Ionene)	1341	1349 ^[28]	B	0.4	nd	1,2
(<i>E</i>)-2-Undecenal	1355	1357	A	nd	0.1	1,2
(<i>Z</i>)-β-Damascenone	1363	1361	IT	0.6	nd	1,2
Decanoic acid	1364	1364	A	nd	0.1	1,2,3
(<i>E</i>)-β-Damascenone	1380	1383	IT	nd	0.9	1,2
1-Tetradecene	1388	1388	A	nd	0.1	1,2
Tetradecane	1400	1400	A	0.6	0.2	1,2,3
Dodecanal	1410	1408	A	0.4	0.4	1,2
β-Caryophyllene	1418	1417	S	1.0	0.2	1,2,3
Coumarin	1432	1432	PP	0.8	nd	1,2
2-Hydroxy-4-methoxyacetophenone (=Paeonol)	1437	1438 ^[30]	B	0.5	nd	1,2
Geranylacetone	1446	1453	IT	1.6	0.9	1,2
Allo-aromadendrene	1458	1458	S	nd	0.1	1,2
2,6,10,14-Tetramethylheptadecane	1461	1464 ^[31]	A	nd	1.0	1,2
Dodecan-1-ol	1473	1469	A	nd	2.0	1,2
(<i>E</i>)-β-Ionone	1479	1487	IT	3.3	nd	1,2
Tridecan-2-one	1495	1495	A	0.3	0.1	1,2
Pentadecane	1500	1500	A	1.5	t	1,2,3
Tridecanal	1511	1509	A	1.3	0.2	1,2
(<i>E</i>)-Nerolidol	1560	1561	S	nd	5.9	1,2,3
Dodecanoic acid	1565	1565	A	nd	0.3	1,2,3
(<i>Z</i>)-3-Hexenyl benzoate	1568	1565	B	0.2	t	1,2
(<i>Z</i>)-dihydro-apofarnesol	1572	1571	S	0.8	0.2	1,2
α-Cedrene epoxide	1578	1574	S	0.6	nd	1,2
Germacrene-D-4-ol	1579	1574	S	nd	0.4	1,2
1-Hexadecene	1589	1588	A	0.2	nd	1,2
(<i>E</i>)-dehydro-apofarnesol	1590	1590	S	nd	0.3	1,2
Hexadecane	1600	1600	A	0.6	nd	1,2,3
Ledol	1605	1602	S	0.2		1,2
Humulene epoxide II	1608	1608	S	nd	t	1,2
Dodecyl acetate	1609	1607	A	nd	2.0	1,2
Tetradecanal	1613	1611	A	1.8	0.3	1,2
α-Cadinol	1651	1652	S	nd	0.2	1,2
Tetradecan-1-ol	1668	1671	A	nd	0.2	1,2
Pentadecan-2-one	1696	1697	A	nd	0.1	1,2
Heptadecane	1700	1700	A	0.6	0.1	1,2,3
Pentadecanal	1716	1713 ^[32]	A	18.7	2.1	1,2
Tetradecanoic acid	1768	1764 ^[31]	A	nd	2.7	1,2
Drimenone	1792	1792	S	nd	t	1,2
Octadecane	1800	1806	A	0.2	t	1,2,3
Hexadecanal	1817	1819 ^[33]	A	0.6	0.1	1,2
6,10,14-Trimethyl-2-pentadecanone (=Hexahydrofarnesylacetone)	1843	1846 ^[32]	A	2.3	1.0	1,2
Pentadecanoic acid	1858	1866 ^[34]	A	nd	0.5	1,2
(5 <i>E</i> ,9 <i>Z</i>)-farnesylacetone	1887	1886	IT	nd	0.2	1,2
Methyl linolenate	1889	1893 ^[27]	A	6.0	nd	1,2
Nonadecane	1900	1900	A	0.1	0.2	1,2,3

(5E,9E)-Farnesylacetone	1909	1913	IT	0.3	0.2	1,2
Heptadecanal	1920	1920 ^[35]	A	0.5	0.1	1,2
Methyl hexadecanoate	1926	1921	A	nd	0.2	1,2
Cyclohexadecanolide	1938	1933	Misc	nd	0.8	1,2
(E)-Phytol	1943	1942	D	nd	t	1,2
Isophytol	1945	1946	D	0.1	nd	1,2
Hexadecanoic acid	1958	1959	A	1.3	19.7	1,2,3
1-Eicosene	1989	1987	A	0.1	nd	1,2
Ethyl hexadecanoate	1990	1992	A	nd	0.2	1,2
Eicosane	2000	2000	A	t	nd	1,2,3
(E,E)-Geranyl linalool	2021	2026	D	0.1	nd	1,2
Octadecanal	2044	2037 ^[34]	A	nd	0.4	1,2
Methyl linoleate	2094	2095	A	nd	0.2	1,2
Heneicosane	2100	2100	A	0.1	0.1	1,2,3
(Z)-phytol	2107	2113 ^[31]	D	7.8	1.0	1,2
Linoleic acid	2130	2132	A	nd	3.4	1,2,3
Docosane	2200	2200	A	t	nd	1,2,3
Tricosane	2300	2300	A	0.1	0.1	1,2,3
Tetracosane	2400	2400	A	0.1	0.1	1,2,3
Pentacosane	2500	2500	A	0.1	0.8	1,2,3
Hexacosane	2600	2600	A	t	0.2	1,2,3
Heptacosane	2700	2700	A	t	3.1	1,2,3
Octacosane	2800	2800	A	nd	0.3	1,2,3
Nonacosane	2900	2900	A	nd	2.1	1,2,3
Total identified				87.9	89.2	
Number of compounds				96	109	

^aCompounds listed in order of elution from poly (5% phenyl 95% methylsiloxane) column.

^bRI = Retention index relative to C₈-C₃₂ n-alkanes on the Poly (5% phenyl 95% methylsiloxane) column. ^cRI= Lit.^[26].

^dMethod: 1 = Retention index on poly (5% phenyl-95% methylsiloxane) column; 2 = MS spectra; 3 = Standard; t: traces (<0.05%); nd: not detected.

Table 2: The chemical class distribution in the essential oils of *Gliricidia sepium*.

	Leaves		Flowers	
	Percentage	NC	Percentage	NC
Aliphatics (A)	54.9	58	58.9	67
Hydrocarbons	4.7	17	8.5	16
Alcohols	7.3	13	12.4	15
Aldehydes	29.9	13	7.1	17
Ketones	4.4	8	1.5	6
Acids	1.8	2	26.7	6
Esters	6.8	5	2.7	7
Terpenoids	28.1	26	25.8	35
Monoterpenoids (M)	10.7	15	15.5	21
Sesquiterpenoids (S)	3.6	4	7.3	9
Diterpenoids (D)	8.0	3	1.0	2
Irregular terpenoids (IT)	5.8	4	2.0	3
Aromatics	5.4	9	2.2	4
Benzenoids (B)	5.0	8	2.2	4
Phenylpropanoids (PP)	0.8	1		
Miscellaneous (Misc.)	0.3	3	1.1	2

4. Conclusions

The leaf and flower essential oils of *Gliricidia sepium* were dominated by aliphatic compounds (54.9-58.9%). Hexadecanoic acid (19.7%), pentadecanal (18.7%) and nonanal (5.1%) were found to be the main constituents. Among terpenoids, the flower oil yields myrtenol (7.7%) and (E)-nerolidol (5.9%). The composition of leaf and flower essential oils of *Gliricidia sepium* from Chomes, Pacific coast of Costa Rica, appear to be markedly different to the composition of the oils from samples of the same

morphological parts from cultivated material at Kerala, India.

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6. References

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