CHEMICAL COMPONENTS OF *PLUMIERIA ALBA* LINN.

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Plumieria alba Linn. (Family: Apocynaceæ) is a small tree generally found to grow along with *P. acutifolia* Poir. in many parts of India. The plant was originally a native of the West Indies but is now naturalized in several regions of India. The root bark is purgative, alterative and detergent. An extract of the root bark is used internally and externally for syphilitic ulcers.[1] No chemical work on this plant has been reported so far. The chemical examination of its bark has therefore been undertaken and the results are recorded herein.

The powdered bark was extracted with petroleum ether in the cold, then with ether and finally with hot alcohol. The extracts were examined separately. The petroleum ether extract residue was chromatographed on alumina. From the eluates by crystallization from solvents and solvent mixtures a mixture of α- and β-amyrin acetates, a mixture of α- and β-amyrins, β-sitosterol and an unidentified minor component were obtained in the order given. The ether extract yielded three unidentified minor components besides scopoletin.

The alcoholic extract was treated with freshly precipitated lead hydroxide and the filtered extract was concentrated to an aqueous syrup which was extracted with ether, chloroform and chloroform-alcohol (2:1). From the alkali-soluble portion of the ether extract scopoletin was again obtained while the chloroform-alcohol (2:1) extract yielded the glucoside plumieride. From the mother liquors of scopoletin another minor component could be obtained after acetylation.

The mixture of α- and β-amyrins was resolved through their anisates by fractional crystallization from acetone and chloroform-alcohol. The individual amyrins were obtained by hydrolysing the pure anisates.

β-Sitosterol was characterized by its m.p., optical rotation, colour reactions and analysis and this was confirmed by the preparation and characterization of its acetate. As the sterol could be obtained directly by chromato-

graphy of the petroleum ether extract it is evidently present in the bark as the free sterol and not as its ester.

Scopoletin was identified by its m.p., fluorescence in alkaline and alcoholic solution, ferric colour and analysis. This was confirmed by the preparation and characterization of its methyl ether. Further the mixed m.p. between the original compound and authentic scopoletin was found to be undepressed. Scopoletin and its glucoside, scopolin (fabiatrin, murrayin) have been isolated from a number of species of flowering plants belonging to the families, Solanaceæ, Gramineæ, Rosaceæ, Rutaceæ, Loganiaceæ, Convolvulaceæ, Compositæ and Apocynaceæ.

Plumieride was characterized by its physical and chemical properties and by a direct comparison (mixed m.p. and colour reactions) with authentic plumieride obtained from the bark of *P. acutifolia* Poir.[2] Plumieride (originally called agoniadin) was first isolated by Peckolt[3] from the bark of *Plumieria lancifolia* and subsequently by a number of workers[4–7] from the bark of *P. acutifolia*. Its constitution including stereochemistry has been elucidated by Schmid and coworkers.[7–8]

EXPERIMENTAL

The bark was collected from trees growing round about the Andhra University campus in Waltair.

The powdered bark (2 kg.) was extracted with petroleum ether (3×5 l) in the cold. The petroleum ether-free marc was extracted with ether (3×4 l). The ether-free marc was extracted with 90% alcohol by refluxing it with the solvent (2×5 l) in a boiling water-bath for 4 hours each time. After these extractions the marc was no more bitter and was discarded.

EXAMINATION OF THE PETROLEUM ETHER EXTRACT

The semi-solid residue (58 g.) obtained by distillation of the solvent was chromatographed by dissolving in petroleum ether (300 ml.), pouring over a 300 g. column of alumina and eluting with 600 ml. portions of solvents and solvent mixtures. The results of the chromatography are briefly summarised in Table I.

Fractions 1 and 2.—The semi-solid residue (45·8 g.) slowly deposited crystalline rosettes. It proved difficult to separate the crystals as any solvent added to dissolve the matrix dissolved the crystals also. The mixture was repeatedly extracted with hot 80% alcohol. From the alcoholic extracts a colourless powder, m.p. 200–10° (softening at 170–80°), separated. It was

TABLE I

Fraction No	Eluate	Weight of residue g.	Substance obtained	M.p. after one crystallization
1-2	Petroleum ether	45·8	Amyrin acetate	170–80° (softening) 200–210°
3–5	Petroleum ether-Benzene (9 : 1), (3 : 1) and (1 : 1)	6·02	Amyrin mixture	172–180°
6	Benzene	0·78	β-sitosterol	136–38°
7	Benzene-Chf (19 : 1)	0·08	Amorphous	
8	Benzene-Chf (9 : 1)	0·34	Minor component 1	208–10°
9–10	Benzene-Chf (3 : 1) and (1 : 1)	1·52	Amorphous	
11	Chloroform	0·05	Amorphous	

twice crystallized from alcohol and once from ethyl acetate when fine colourless needles of amyrin acetate, m.p. 195° (softening) 210–15° were obtained (0·3 g.). The m.p. could not be improved by further crystallizations. The substance gave triterpenoid colour reactions.

$[\alpha]_D^{29} = +78{\cdot}1° \pm 3°$ ($c = 1{\cdot}012$ in benzene).

[Found: C, 82·4; H, 11·8; —$COCH_3$, 8·6%. $C_{32}H_{52}O_2$ (amyrin acetate)[5] requires C, 82·0; H, 11·2; —$COCH_3$ (1), 9·2 %.]

That the substance was an acetate was further qualitatively confirmed by hydrolysing it with alcoholic potash, acidifying with dilute sulphuric acid and distilling the mixture. The distillate gave with lanthanum nitrate reagent[9] a blue colour characteristic of acetic acid.

α-β-Amyrin mixture from the mixed acetate.—The large quantity of mother liquors of amyrin acetate (44·9 g.) was saponified with N/2 benzene-alcoholic potash and the amyrin (18·3 g.) obtained from the unsaponifiable matter was crystallized from alcohol when colourless needles, m.p. 175–82°, were obtained. The substance gave positive Libermann-Burchard and Salkowski reactions.

$[\alpha]_D^{30} = +93{\cdot}7° \pm 3°$ ($c = 1{\cdot}104$ in benzene). [Found: C, 84·1; H, 11·4%. $C_{30}H_{50}O$ (amyrin) requires C, 84·5; H, 11·8%.]

Preparation of anisate.—Amyrin (3·3 g.) obtained in the above experiment was anisoylated with absolute pyridine (30 ml.) and freshly distilled anisoyl chloride (3 ml.). The crude anisate (4·2 g.) sintered at 160° and melted at 180–90°. It was chromatographed over 120 g. of alumina employing 400 ml. proportions of solvents and solvent mixtures for elution. No chromatographic fraction was completely pure. On repeated fractional crystallizations from acetone, each was resolved into two to three crops. The most soluble crop from each eluate gave α-amyrin anisate (total 2·9 g.), needles from chloroform-alcohol, m.p. 192–94°, $[\alpha]_D^{29} = +101{\cdot}6° \pm 2°$ ($c = 1{\cdot}215$ in benzene). [Found: C, 81·8; H, 10·6; $-OCH_3$, 5·3%. $C_{38}H_{56}O_3$ (amryin anisate) requires C, 81·4; H, 10·1; $-OCH_3$ (1), 5·5%.]

The crops melting in the range of 210–18°, obtained in the above fractionations, were all mixed and fractionally crystallized from acetone, rejecting the more soluble portions at each stage. After repeated recrystallizations in this manner, a fraction melting at 248–50° was obtained (0·6 g.) which corresponded to β-amyrin anisate in its properties. $\alpha_D^{31} = +95{\cdot}7° \pm 2°$ ($c = 1{\cdot}213$ in benzene). [Found: C, 82·0; H, 10·3; $-OCH_3$, 6·0%. $C_{38}H_{56}O_3$ (amyrin anisate) requires C, 81·4; H, 10·1; $-OCH_3$ (1), 5·5%.]

α-Amyrin from its anisate.—α-Amyrin anisate (1 g.) (obtained as above) was saponified with benzene-alcoholic potash. The solvents were removed under reduced pressure and the residue diluted with water when a solid separated out. It was filtered, washed with water, dried and twice crystallized from alcohol when colourless needles, m.p. 182–84°, were obtained (yield: 0·71 g.). $[\alpha]_D^{29} = +89{\cdot}8° \pm 2°$ ($c = 1{\cdot}026$ in benzene). [Found: C, 84·2; H, 12·0%. $C_{30}H_{50}O$ (amyrin) requires C, 84·5; H, 11·8%.] The aqueous filtrate after acidification and ether extraction gave anisic acid (m.p. and mixed m.p. 183° and 183–84° respectively).

β-Amyrin from its anisate.—β-Amyrin anisate (0·3 g.) obtained as described above) was hydrolysed similarly to give pure β-amyrin (0·18 g.), long needles from alcohol, m.p. 194–96°. $[\alpha]_D^{29} = +97{\cdot}8° \pm 3°$ ($c = 0{\cdot}926$ in benzene). [Found: C, 84·7; H, 12·1%. $C_{30}H_{50}O$ (amyrin) requires C, 84·5; H, 11·8%.]

Mixture of free α- and β-amyrins from the chromatogram of the petroleum ether extract.—Fractions 3–5 of Table I were crystallized from petroleum ether. In all the three cases colourless needles having a melting range of 170–82° were obtained (yield 5·1 g.). $[\alpha]_D^{29} = +92{\cdot}7° \pm 2°$ ($c = 1{\cdot}112$ in benzene). [Found: C, 84·2; H, 12·2%. $C_{30}H_{50}O$ (amyrin) requires

C, 84·5; H, 11·8%.] The substance was free from acetyl (lanthanum nitrate test). 1 g. was anisoylated and the crude anisate was fractionally crystallized from acetone as described already when pure α-amyrin anisate, m.p. 191–94° (0·5 g.) and pure β-amyrin anisate, m.p. 249–51° (0·3 g.) were obtained. The anisates on hydrolysis yielded the pure amyrins.

β-Sitosterol.—Fraction 6 of Table I crystallized from petroleum ether as fine needles, m.p. 136–38° (yield 0·62 g.). Recrystallization from alcohol gave plates, m.p. 138–40°. It gave positive Liebermann-Burchard and Salkowski reactions. $[\alpha]_D^{30} = -31{\cdot}8° \pm 2°$ ($c = 1{\cdot}101$ in chloroform). [Found: C, 83·7; H, 12·7%. $C_{29}H_{50}O$ requires C, 84·0; H, 12·2%.]

The acetate prepared using sodium acetate and acetic anhydride crystallized from alcohol as fine needles, m.p. 128–30°. $[\alpha]_D^{31} = -37{\cdot}9° \pm 2°$ ($c = 1{\cdot}108$ in chloroform). [Found: C, 81·6; H, 12·1%. $C_{31}H_{52}O_2$ (β-sitosterol acetate) requires C, 81·5; H, 11·5%.]

Minor component 1.—Fraction 8 of Table I crystallized from petroleum ether yielding a colourless substance, m.p. 208–10° (*ca.* 10 mg.). It gave positive sterol colour reactions.

Examination of the Ether Extract

From the greenish yellow extract the solvent was removed by distillation. During this process a colourless substance (minor component 2) separated which was filtered and washed with petroleum ether and ether. The greenish brown residue from the filtrate weighed 16 g.

Minor component 2.—This substance (0·2 g.), m.p. 270–82°, was very sparingly soluble in the common organic solvents, but was soluble in pyridine. It could be obtained only as granules from methanol, m.p. 265° (softening), 280–84° (decomp.). It answered the usual sterol and triterpenoid colour reactions. $[\alpha]_D^{30} = +21{\cdot}2° \pm 3°$ ($c = 0{\cdot}926$ in pyridine). [Found: C, 70·7; H, 10·6%. $C_{30}H_{52}O_6$ (probable formula) requires C, 70·8; H, 10·3%.]

Partition of the mother liquors of minor component 2 *and isolation of minor components* 3, 4 *and substance S.*—The residue (16 g.) was partitioned between petroleum ether (600 ml.) and 70% methanol (400 ml.). The petroleum ether layer was further extracted with 2 portions of 250 ml. each of 70% methanol. The aqueous methanolic extracts were passed in succession through two separating funnels containing 500 ml. of petroleum ether in each. All the petroleum ether extracts were united, dried over sodium sulphate and the

solvent removed by distillation. The greenish oily residue (fraction X) weighed 13·5 g.

The 70% methanolic extracts were united and concentrated to an aqueous liquid and extracted with chloroform (4×50 ml.). The extracts were passed through three separating funnels containing 15 ml. of 2 N sodium carbonate in each and then through two separating funnels containing 10 ml. of water in each. The chloroform extracts were dried over sodium sulphate and the solvent removed under vacuum. The neutral amorphous residue weighed 0·342 g. It was not examined further.

The alkaline solutions (brown with blue fluorescence) and the aqueous washings were united, acidified at 0° with hydrochloric acid and extracted with chloroform (4×30 ml.). The chloroform extract residue (fraction Y) weighed 1·42 g.

Fraction X.—The residue when chromatographed over alumina gave minor components 3 and 4 on elution with petroleum ether-benzene (4 : 1) and (1 : 1) respectively.

Minor component 3.—Crystallised from alcohol in short colourless needles, m.p. 166–68° (yield 0·2 g.) and gave positive sterol colour reactions. $[\alpha]_D^{31} = -2·7° \pm 2°$ ($c = 0·912$ in chloroform). [Found: C, 83·9; H, 11·9%. $C_{29}H_{50}O$ (probable formula) requires C, 84·0; H, 12·2%.] Its acetate crystallized from alcohol as small plates, m.p. 127–29°. $[\alpha]_D^{28} = -9·9° \pm 3°$ ($c = 0·718$ in chloroform). [Found: C, 82·0; H, 11·5%. $C_{31}H_{52}O_2$ (monoacetate) requires C, 81·5; H, 11·5.%.]

Minor component 4.—Crystallized from alcohol as pale yellow needles, m.p. 170° (softening), 195–200° (yield 10 mg.). It did not answer sterol colour reactions. [Found: C, 61·5; H, 5·2%. $C_{15}H_{14}O_6$ (probable formula) requires C, 62·1; H, 4·9%.]

Fraction Y.—The residue was extracted with hot petroleum ether, hot benzene and ether until each solvent extracted no more material. The amorphous dark brown material left after these extractions was discarded. From the residues of the benzene- and ether-solubles a light brown susbtance (55 mg.), m.p. 198–200°, was obtained after crystallization from petroleum ether (substance S, see later).

ALCOHOL EXTRACT

The combined dark brown alcoholic extract (*ca.* 10 l.) was concentrated under reduced pressure to about 4 l., shaken with freshly precipitated lead

hydroxide (obtained from 500 g. of lead acetate) on a machine for 2 hours, filtered through a thin bed of kieselguhr and the precipitate washed well with alcohol. Lead present in the filtrate was removed by passing hydrogen sulphide and filtering. The lead free filtrate was further concentrated to an aqueous syrup (*ca.* 500 ml.). This was left in the ice-chest for a number of days. No solid separated. It was then extracted with ether (5×300 ml.), chloroform (3×250 ml.) and chloroform-alcohol (2 : 1) (4×300 ml.). The three extracts were examined separately.

The ether extracts were washed with water (20 ml.), 2 N sodium carbonate (3×20 ml.) and water (2×15 ml.), dried over sodium sulphate and the solvent removed by distillation. The neutral amorphous bitter residue (3·5 g.) was not examined further. The sodium carbonate solutions (brown with blue fluorescence) and the subsequent aqueous washings were united, acidified with hydrochloric acid (1 : 1) at 0° and extracted with chloroform (3×40 ml.). The chloroform extract residue was given the same treatment as described under fraction Y when some more of the substance S (105 mg.) could be obtained.

The chloroform and chloroform-alcohol (2 : 1) extracts were washed with water, 2 N sodium carbonate and water, dried over sodium sulphate and the solvents removed under vacuum. The chloroform extract yielded a brown semi-solid residue (3·2 g.) which could not be crystallized and was not examined further.

Isolation of plumieride.—The chloroform-alcohol (2 : 1) extract residue (42 g.) was crystallized from methanol-acetone when colourless needles separated out. They were filtered, washed with acetone and dried, m.p. 152–56° (hydrated substance) (yield 13·5 g.). The substance was recrystallized alternately from methanol-acetone and methanol-benzene and thus colourless prisms, m.p. 219–22° (anhydrous substance) were obtained. It gave positive Molisch test and negative Legal and Keller-Kiliani reactions. With concentrated sulphuric acid it gave an yellow colour which changed into deep yellow and pink (after 2 hours). $[\alpha]_D^{29} = -104{\cdot}7° \pm 2°$ ($c = 0{\cdot}841$ in water). [Found on the anhydrous substance: C, 52·9; H, 6·1; —OCH_3, 6·3%. $C_{21}H_{26}O_{12}$ (plumieride) requires C, 53·6; H, 5·6; —OCH_3 (1), 6·6%.] Mixed m.p. with authentic plumieride obtained from *P. acutifolia*[2] was undepressed.

Substance S (*scopoletin*).—The crystalline substance obtained from the fraction Y of the ether extract and the substance obtained from the ether solubles of the main alcohol extract had identical properties and hence they were mixed (total 160 mg.) and twice crystallized from chloroform-ether when

colourless long needles (130 mg.) were obtained. The substance (plates from alcohol) changed its crystal form at 180° and melted at 204–06°. Its alcoholic and alkaline solutions exhibited blue fluorescence. With alcoholic ferric chloride it gave a green colour. [Found: C, 63·0; H, 4·4; $-OCH_3$, 15·7%. $C_{10}H_8O_4$ (scopoletin) requires C, 62·5; H, 4·2; $-OCH_3$ (1), 16·2%.] Mixed m.p. with an authentic sample of scopoletin was undepressed.

Scopoletin methyl ether.—The substance (50 mg.) was methylated with diazomethane and the product crystallized twice from alcohol when fine long yellow needles separated, m.p. 144–45°. It gave no colour with ferric chloride. [Found: C, 64·4; H, 5·4; $-OCH_3$, 29·5%. $C_{11}H_{10}O_4$ requires C, 64·1; H, 4·9; $-OCH_3$ (2), 30·1%.]

Acetate of minor component 5.—The brown semi-solid mass (0·52 g.) obtained by evaporation of the solvent from the mother liquors of substance S (both from ether extract and alcohol extract) was acetylated with anhydrous sodium acetate and acetic anhydride. The resulting dark pasty product was dissolved in alcohol and treated with activated charcoal and filtered. The residue obtained by evaporation of the alcoholic extract was digested with ether. The ether solution on evaporation gave a very small quantity of scopoletin acetate, m.p. 174–77° and a small amount of a colourless powder (45 mg.), m.p. 200–06°. The latter was recrystallized from alcohol when fine colourless plates, m.p. 210–14°, were obtained. The substance was soluble in dilute aqueous alkali but gave no fluorescence. It was soluble even in aqueous sodium bicarbnoate, though not very readily. From both sodium hydroxide and bicarbonate solution it could be reprecipitated by the addition of acid. It gave no colour with alcoholic ferric chloride. With concentrated sulphuric acid it gave an yellow colour. The substance was free from methoxyl. [Found: C, 62·3; H, 4·9; $-COCH_3$, 26·9%. $C_{17}H_{14}O_7$ (probable formula) requires C, 61·8; H, 4·3; $-COCH_3$ (2), 26·1%.] On hydrolysis with methanolic hydrochloric acid it gave a very small quantity of a substance, m.p. 135–40° (impure) which gave greenish brown colour with alcoholic ferric chloride.

SUMMARY

The chemical examination of the bark of *Plumieria alba* Linn. by extraction with solvents and fractionation is described. Amyrin acetate (α, β-mixture), amyrin (α, β-mixture), β-sitosterol, scopoletin and plumieride have been isolated and characterized. Besides these, four minor components have been obtained in yields which did not permit closer examination.

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