first absorbed with the homologous antigen I showed that the homologous antigen precipitated all of the antibody, since the supernatant liquor gave no further precipitation reaction when 30 mcg. of I was added.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 16, 1971, from the *Department of Microbiology, Division of Immunology*, and the *Department of Biochemistry, University of Alabama Medical School, Birmingham, AL 35233*

Accepted for publication February 16, 1972.

Supported by the National Science Foundation, The Hartford Foundation, and the State of Alabama.

The technical assistance of Miss Nora Tsang is gratefully acknowledged.

Chemical Constituents of *Salmalia malabarica* Schott and Endl. Flowers

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Abstract □ *Salmalia malabarica* Schott and Endl. flowers have been shown to contain the β-D-glucoside of β-sitosterol, free β-sitosterol, hentriacontane, hentriacontanol, traces of an essential oil, kaempferol, and quercetin.

Keyphrases □ *Salmalia malabarica*—chemical constituents of flowers □ Medicinal plants—*Salmalia malabarica* flowers, chemical characterization

Salmalia malabarica Schott and Endl. (N. O. Malvaceae) is a tall deciduous tree distributed throughout the hotter parts of India and Ceylon and is known to possess medicinal properties. The crimson-red flowers are highly valued in the treatment of leucorrhoea and hemorrhoids and also are used externally for boils, sores, and itch (1, 2). Since no work concerning this plant has been reported in the literature, except for the structure of the gum (3, 4), it was considered of interest to undertake a detailed chemical investigation of the flowers.

The alcoholic extract of the flowers yielded hentriacontane, hentriacontanol, the β-D-glucoside of β-sitosterol, free β-sitosterol, an essential oil, quercetin, and kaempferol. The essential oil was obtained on steam distillation of the alcoholic extractive. The two flavonols were separated by the method of Elsissi and Saleh (5). The isolated compounds were identified on the basis of their physicochemical properties and comparison with authentic samples.

EXPERIMENTAL[1]

Air-dried coarsely powdered flowers (2 kg.) were exhaustively extracted with alcohol (95%) by cold percolation. The solvent (22 l.) was removed under reduced pressure. The alcoholic extractive (18 g.) was successively extracted with petroleum ether, b.p. 40–60° (3 l.), and benzene (2 l.).

Petroleum Ether Extract—On concentration, a white residue, A (900 mg.), was obtained, which was filtered and washed with petroleum ether and ether.

β-D-Glucoside of β-Sitosterol—Residue A crystallized from methanol (charcoal) as colorless needles (250 mg.), m.p. 294–296°; $[\alpha]_D^{30}$ −37.4° (c, 0.9480, pyridine). The compound gave a positive Liebermann–Burchard (L–B) color reaction, showing a typical sequence of colors (pink–violet–blue–green), and also responded to Molisch's test. The IR spectrum showed characteristic peaks at 3450, 1355, and 1370 cm.$^{-1}$.

Anal.—Calc. for $C_{35}H_{60}O_6$: C, 72.84; H, 10.48. Found: C, 72.44; H, 10.68.

Acetate—The acetate was prepared by heating the glucoside with acetic anhydride in the presence of fused sodium acetate (5 hr., 130°). On crystallization from methanol, it was obtained as glistening plates, m.p. 166–168°; $[\alpha]_D^{30}$ −28.8° (c, 1.2, pyridine), and analyzed for $C_{43}H_{68}O_{10}$. Mixed melting point with a known sample of tetraacetate of the β-D-glucoside of β-sitosterol was undepressed.

Hydrolysis of β-D-Glucoside of β-Sitosterol—The glucoside was refluxed with methanolic hydrochloric acid (7%, 8 hr.). The aglycone was worked up as usual and crystallized from ethanol as colorless needles, m.p. 135–137°; $[\alpha]_D^{28}$ −35° (c, 1.2, $CHCl_3$). It gave positive L–B and Salkowski color reactions for sterol. Mixed melting point with an authentic specimen of β-sitosterol showed no depression.

Anal.—Calc. for $C_{29}H_{50}O$: C, 84.07; H, 12.1 Found: C, 83.7; H, 12.3.

β-Sitosterol Acetate—This was obtained as colorless needles (acetone), m.p. and mixed m.p. 128–129°; $[\alpha]_D^{30}$ −38° (c, 1.0, $CHCl_3$).

The sugar moiety in the hydrolysate was confirmed as glucose by paper partition chromatography and preparation of osazone.

Benzene Extract—The filtrate, after the removal of Residue A, was mixed with the benzene extract and the solvents were removed by distillation. The residue, on steam distillation, gave an essential oil (0.003%), having $n_D^{31°}$ 1.428 and $d^{31°}$ 0.8697. The essential oil was not examined further due to its poor yield. The nonvolatile fraction was chromatographed over Brockmann's alumina column using hexane, benzene, and their mixtures as eluent.

Hentriacontane—This was obtained from the hexane eluate as colorless shining plates (800 mg.), m.p. 67–68°. It was optically inactive, and IR spectra showed the absorption band at 731 cm.$^{-1}$ (alkane). Identity of the compound was confirmed on the basis of a

[1] The species determination was done by Dr. S. R. Gupta, Indian Grassland & Fodder Research Institute, Jhansi, India. The plant was identified on February 4, 1970; Accession Number 1, I.G.F.R.I. Herbarium Forest species 404.

mixed melting-point determination with a known sample and their identical IR spectra.

Anal.—Calc. for $C_{31}H_{64}$: C, 85.31; H, 14.68. Found: C, 85.52; H, 14.46.

Hentriacontanol—Elution with a benzene–hexane mixture (1:1) yielded a saturated and optically inactive compound (540 mg.), m.p. 82–84°, identified as hentriacontanol. The IR spectrum showed peaks at 3550 (—OH) and 800 cm.$^{-1}$ (—CH_2).

Anal.—Calc. for $C_{31}H_{64}O$; C, 82.30; H, 14.16. Found: C, 82.42; H, 14.32.

Hentriacontanol Acetate—This was prepared as usual and crystallized from acetone as colorless flakes, m.p. 74–76°. It was analyzed for $C_{33}H_{66}O_2$.

β-Sitosterol—The benzene eluate furnished free β-sitosterol (350 mg.), m.p. 135–137°, identified *vide supra*.

The alcoholic extractive was finally extracted with hot distilled water (2 l.). The aqueous extract was treated with lead acetate solution (5%) and filtered. The precipitated lead complex was suspended in alcohol and decomposed with hydrogen sulfide gas. The sulfide was filtered, and alcohol was removed under reduced pressure. Because it did not yield any crystalline product, the concentrate was hydrolyzed with alcoholic hydrochloric acid (7%) and worked up as usual. The hydrolysate showed two spots on the TLC plate, corresponding to kaempferol and quercetin.

Kaempferol—The hydrolysate was dissolved in aqueous borax solution (0.5%) and exhaustively extracted with ethyl acetate. The ethyl acetate fraction, on crystallization from dilute ethanol, gave a yellow crystalline substance (350 mg.), m.p. 274–276°. This was identified by mixed melting point and cochromatography with an authentic sample of kaempferol.

Anal.—Calc. for $C_{15}H_{10}O_6$; C, 62.9; H, 3.49. Found: C, 62.68; H, 3.38.

It formed a tetraacetate as silky needles, m.p. 181–183° (fused sodium acetate and acetic anhydride; 4 hr., 130°) and tetramethyl ether, m.p. 150–152° (dimethyl sulfate and anhydrous potassium carbonate in the presence of acetone).

Quercetin—The remaining borax-soluble fraction was decomposed with dilute hydrochloric acid and extracted with solvent ether. On removal of the solvent and crystallization from dilute methanol, a microcrystalline yellow substance (700 mg.), m.p. 308–310°, was obtained; UV absorption: $\lambda_{max.}^{ethanol}$ 275 (log ε 4.33) and 375 nm. (log ε 4.32).

Anal.—Calc. for $C_{15}H_{10}O_7$: C, 59.60; H, 3.31. Found: C, 59.5; H, 3.42.

It showed no depression in melting point with a known sample of quercetin. The compound formed a pentaacetate, m.p. 193–195°, and a pentamethyl ether, m.p. and mixed m.p. 152–153°.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 19, 1971, from the *Department of Chemistry, Catholic University of America, Washington, D.C., and the †Indian Veterinary Research Institute, Izatnagar, India.

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Naturally Occurring and Synthetic β-Carbolines as Cholinesterase Inhibitors

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Abstract □ Inhibitory activities of a number of naturally occurring and synthetic aromatic and tetrahydro-β-carbolines toward true and pseudocholinesterases were investigated. The potencies of the active compounds were measured and compared with those of physostigmine by their ability to protect acetylcholine from the cholinesterase (assayed on frog *rectus abdominis muscle*) and by using the chromodacryorrhea test in albino rats. Some quaternary aromatic β-carbolines were found to be about one-sixth as potent as physostigmine. The inhibitory potencies were nearly the same against acetylcholinesterase and pseudocholinesterase. The tertiary aromatic β-carbolines showed only weak activity, while the tetrahydro-β-carbolines lacked this activity. Influence of indole-*N*-methylation on the inhibitory activity was greater with the quaternary β-carbolines than with the tertiary series. The activity also depended on the degree of extended conjugation of the inhibitor molecule. Other factors involved in the anticholinesterase activities of the β-carbolines were examined, and a plausible mechanism of action was proposed.

Keyphrases □ β-Carbolines, natural and synthetic—as cholinesterase inhibitors, pharmacological evaluation, mechanism of action □ Cholinesterase inhibitors—pharmacological evaluation of naturally occurring and synthetic β-carbolines, mechanism of action □ Anticholinesterase activity—pharmacological evaluation of naturally occurring and synthetic β-carbolines, mechanism of action

Although considerable work has been done on naturally occurring anticholinesterases (1), comparatively fewer investigations have been made so far on the possible anticholinesterase activities of β-carbolines. In two recent papers, we sought to rationalize the reported (2) uses of the bark and fruit extracts of two *Desmodium* species—*viz.*, *D. pulchellum* and *D. gangeticum* (Family Leguminosae), in the Indian system of medicine for eye diseases and certain intestinal malfunctions with the potent anticholinesterase activities of their total alkaloids (3, 4). The present paper reports the anticholinesterase activities of some individual β-carbolines isolated from the above plant parts and those of their synthetic analogs. In addition, anticholinesterase activities of a number of complex indole alkaloids, containing an aromatic β-carboline