

colour with alc. FeCl_3 and reduced Tollen's and Fehling's reagents. On acetylation with acetic anhydride and anhydrous sodium acetate, it gave a penta acetate, m.p. $151-52^\circ$ and on methylation with dimethyl sulphate and potassium carbonate for 4 hr. in anhydrous acetone medium, a tetramethyl ether, m.p. $153-54^\circ$. These data agreed well with those of (-) epicatechin^{3,4} and the identity of the compound was further confirmed by PC, TLC (single spot), UV, IR and NMR data.

The more soluble fraction obtained from the mother liquor after the removal of (-) epicatechin was almost colourless. On recrystallisation it came out as needles, m.p. $96-98^\circ$ (anhydrous: $176-77^\circ$), $[\alpha]_D^{30} + 16.8$ (yield, 4%). It exhibited all the properties of a flavan-3-ol. Its acetate melted at $130-32^\circ$, benzoate, $170-71^\circ$ and methyl ether (complete methylation, 36 hr.), $94-95^\circ$ all pointing towards the identity as (+) catechin^{3,4}. This identification was also confirmed by UV, IR and NMR data. The proanthocyanidins were studied in the subsequent acetone extract and leucocyanidin and leucopelargonidin as reported by Mathew⁵ could be identified.

Recently, Mathew and Parpia⁶ have identified the polyphenols of cashew kernel testa (50% aqueous acetone extract) by paper chromatography to be (+) catechin, (-) epicatechin, proanthocyanidins, leucocyanidin and leucopelargonidin in agreement with our results. They have also suggested that the darkening of cashewnuts during processing may be due to the interaction of polyphenols with iron.

The isolation of catechins from the cashewnut testa in such high yields is significant from the point of view of biosynthesis of polyphenols of *A. occidentale*, taken in conjunction with our earlier isolation of ethyl gallate¹ in significant amounts from the flowers; it points to a possible conversion of a C_6-C_1 system in the flower to a $C_6-C_3-C_6$ polyphenol in the fruit.

We are grateful to Prof. T. R. Govindachari, Director, Ciba Research Centre, Bombay-63, for UV, IR and NMR data and Drs. A. G. Mathew and H. A. B. Parpia of the C.F.T.R.I., Mysore, for the private communication of their results. Our thanks are due to Mr. K. Vijayan Pillai for the supply of plant material.

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Note added to the proof:

The identity of (-)-epicatechin and (+)-catechin was also confirmed by direct comparison, m.m.p. and co-chromatography with authentic samples from Prof. T. R. Seshadri, to whom we are grateful.

CHEMICAL EXAMINATION OF *SANTALOIDES MINUS*

Santaloides minus (N.O. Connaraceæ) commonly grows as a climbing shrub throughout the country. Its roots are widely used in Ayurvedic medicine as a bitter tonic, in rheumatism and pulmonary complaints.^{1,2} The plant appears to have not been chemically examined to date. They have now been shown to contain β -D-glucoside of β -sitosterol, hentriacontane, β -sitosterol and meso-inositol.

The powdered roots (3 kg.) were exhausted with petroleum ether, b.p. $40-60^\circ$ (4×8 L) by cold percolation. The total extract was concentrated to one litre and kept overnight.

β -D-glucoside of β -sitosterol.—A residue was deposited, which was filtered, washed with petroleum ether and on repeated crystallization from methanol afforded a colourless micro-crystalline product (800 mg.), m.p. $274-76^\circ$, $(\alpha)_D^{30} - 36.2^\circ$ (c, 1.4 pyridine) (Found C, 72.64; H, 10.52; $\text{C}_{35}\text{H}_{60}\text{O}_6$ requires C, 72.84; H, 10.48%). It gave positive Liebermann Burchard colour reaction and Molisch's test. Mixed m.p. with an authentic sample of β -D-glucoside of β -sitosterol was undepressed. It formed tetracetate, m.p. and mixed m.p. $165-67^\circ$, $(\alpha)_D^{30} - 31^\circ$ (c, 1.2 pyridine). The compound on acid hydrolysis with methanolic hydrochloric acid (7%, 8 hr.) furnished β -sitosterol as colourless needles, m.p. and mixed m.p. $134-36^\circ$, $(\alpha)_D^{30} - 33^\circ$, acetate, m.p. and mixed m.p. $128-29^\circ$ and benzoate m.p. $142-44^\circ$.

The solvent was completely removed from the filtrate. The semi-solid residue was saponified with alcoholic sodium hydroxide solution (10%, 4 hr.). The unsaponifiable matter on usual working and chromatography on alumina column yielded two compounds.

Hentriacontane.—On elution with petroleum ether, it was obtained as colourless shining plates (900 mg.), m.p. 66–68°. It was optically inactive and the I.R. spectrum in KBr showed absorption at 720 cm^{-1} (alkane). It analysed for $\text{C}_{31}\text{H}_{64}$ and gave no colouration with tetranitromethane. Mixed m.p. with an authentic sample of hentriacontane remained undepressed.

β -sitosterol was eluted with a mixture of petroleum ether and benzene (4 : 1). It crystallized from acetone as colourless needles (450 mg.), m.p. 135–37° and identified as above.

Meso-Inositol.—Petroleum ether exhausted roots were subsequently extracted with ethanol in cold. The ethanolic extract ($6 \times 4\text{ L}$) was concentrated to 500 ml. and kept at room temperature for 48 hours. A crystalline residue was deposited on the walls of the container. The residue was filtered and on crystallization from methanol (charcoal) afforded colourless crystals, m.p. 221–23° (Found, C, 40.32; H, 6.38; $\text{C}_6\text{H}_{12}\text{O}_6$ requires C, 40.0; H, 6.66%). It was freely soluble in water and was optically inactive. It showed no depression in m.p. with an authentic sample of meso-inositol. The identity was further established by paper chromatography employing butanol, acetic acid and water (4 : 1 : 5), (upper layer) as solvent and aniline hydrogen phthalate as spray giving only one spot (R_f 0.12) identical to meso-inositol. Acetylation yielded inositol hexaacetate, m.p. and mixed m.p. 215–16° (Found C, 49.76; H, 5.82; $\text{C}_{18}\text{H}_{24}\text{O}_{12}$ requires C, 50.00; H, 5.55%).

The authors are highly thankful to the Director, Indian Veterinary Research Institute, Izatnagar, for his keen interest in these investigations.

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June 16, 1969.

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ELECTROPHORETIC SEPARATION OF RNA FROM SNAIL EMBRYOS

TSANEV¹ claimed that agar-gel electrophoresis would clearly separate RNA of various S values. Later Dessev *et al.*² reported separation of ribosomes by ion-agar electrophoresis.

We have now succeeded in obtaining reasonably clear separation of embryonic RNA from *Limnæa* with the help of ion-agar electrophoresis and compared the results with sucrose-density-gradient. It seems that though in many respects ultracentrifugation is a superior method, considerable information can be obtained with this very simple technique of electrophoresis and at least in one respect it is more valuable than density-gradient.

Limnæa eggs and embryos collected from the pond or from vessels in the laboratory (maintained on dry lettuce) were fed with ^{32}P (Trombay) isotope solution at different developmental stages and the RNA was extracted by shaking with hot phenol.³ Marker RNA, i.e., non-radioactive RNA was likewise extracted from *E. coli* and mixed with snail RNA and precipitated by cold alcohol. After centrifugation the RNA pellet was dissolved in 0.1 ml. 20% potassium acetate and 0.1 ml. NaCl (final molarity 0.05). 1.25% ion-agar (dissolved in citrate-phosphate buffer⁴) was melted and 4–5 ml. of this fluid was allowed to set on a microscope slide of usual dimensions. About an hour or so after preparing this layer a groove was cut on the slide and the RNA solution was introduced into the groove and allowed to soak in. After this the two ends of the slide were connected to the baths containing the citrate-phosphate buffer (pH about 8) and the electrophoretic run was allowed to last for 60–75 minutes at a voltage of 350 and constant current of 29 mA.

After this the slide was put in a mixture of phosphate buffer and toluidine blue (final concentration of toluidine blue being 0.1%) in order to stain the RNA bands and wash out the free ^{32}P . After 30–40 minutes of staining and 10–20 minutes of washing in running water the three marker RNA bands (i.e., *E. coli* RNA of 23S, 16S and 4S fractions) would be visible. According to earlier findings¹ the heaviest RNA fraction lies nearest to the origin and as such the individual marker bands can be recognized. Under good conditions the separation between the bands would be as much as 4–5 mm. In such cases, not only the bands but the intermediate regions could be cut out. More often because of diffusion, etc., the clear separation space would be much less. As it has already been found that the γ RNA of *Limnæa* embryos have very nearly the same S values as those of the bacterial γ RNA,³ it was now attempted to cut out the three bands in order to test their relative radioactivity. The cut pieces were washed again overnight in