The combination of counter-current distribution and gel permeation chromatography[11] provides a convenient and exceptionally mild method for dividing a complex extract into groups of materials which are then amenable to handling by more usual techniques. In this case the sterol esters were separated rather cleanly from other ester material and were contaminated only by materials such as solanesol which were easily separable by conventional adsorption chromatography. Analytical gas chromatography showed that cholesteryl esters represented ca. 5% of the phytosteryl ester mixture. Thus these esters represent about 0.005% of the weight of the tobacco[12].

Zusammenfassung. Nach Hydrolyse wurde Cholesterol von einem Sterolester-Bruchteil aus dem «flue-cured» Tabak isoliert und massenspektrometrisch sein Acetat-Derivat einwandfrei festgestellt, obwohl die einzelnen Ester von Cholesterol im Tabak nicht gereinigt oder identifiziert wurden. Zur Isolierung wurden die Zwei-Stufen-Gegenstromverteilung und die Gel-Permeationschromatographie verwendet, 2 brauchbare und wenig aggressive Methoden zur Aufteilung eines komplexen Extraktes in Materialgruppen und nachfolgender gewöhnlicher chromatographischer Bearbeitung.

C. E. Cook, Margaret E. Twine and M. E. Wall

Chemistry and Life Sciences Laboratory, Research Triangle Institute, Research Triangle Park (N. Carolina 27709, USA), 10 July 1967.

[11] Gel permeation chromatography on polystyrene-divinyl benzene polymers has been applied chiefly to polymer mixtures [J. C. Moore, J. Poly. Sci. *2A*, 835 (1964)]. An example of its application to lipids has been recorded by C. L. Tipton, J. W. Paulis and M. D. Pierson, J. Chromat. *14*, 486 (1964). We thank Dr. Tipton for helpful correspondence.

[12] A report of work done under contract with the U.S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract is being supervised by the Eastern Utilization Research and Development Division of the Agricultural Research Service. Mass spectra were obtained by Dr. Maurice Bursey of the University of North Carolina.

Chemical Investigation of *Pluchea lanceolata* I. Isolation of a New Quaternary Base, Pluchine

Pluchea lanceolata, Linn. (N.O. Compositae) (Sanskrit, Hindi, Marathi: *Rasna*; Gujerati: *Rashna*; Bombay: *Kura*, *Sanna*) is a small shrub growing wild in the hotter parts of India, and is used in the Ayurvedic system of medicine in various clinical conditions. It is used as a bitter, a laxative, an analgestic, an antipyretic and a nerve tonic, and for the treatment of rheumatism, dyspepsia and bronchitis[1]. Preliminary pharmacological investigations with the water-soluble fraction of the ethanolic extract of the whole plant of *P.* lanceolata was done by Prasad et al.[2,3]. Detailed chemical investigation of *P. lanceolata* was, therefore, undertaken to isolate the active principles. The petroleum ether extract of *P. lanceolata*, on saponification with strong alkali, extraction with ether, chromatography on aluminium oxide (Brockmann) by elution with different solvents, yielded the following compounds: (1) Compound A, m.p. 92–93 °C (small yield). (2) Compound B, long needles, m.p. 217–221 °C, $[\alpha]_D^{20}$ + 89.2 ($CHCl_3$). Analysis[4] found: C, 83.79, 84,05; H, 11.52, 11.60. Calc. for $C_{30}H_{50}O$: C, 84.44; H, 11.81. *Acetate*, m.p. 238–242 °C, $[\alpha]_D^{20}$ + 91.5, + 95.2 ($CHCl_3$). Analysis found: C, 83.79; H, 11.31. Calc. for $C_{30}H_{49}O.CO.CH_3$: C, 82.05; H, 11.11.

In the Liebermann-Burchard colour reaction, the interface turned violet to brown, and chloroform layer turned pink on standing. No blue or green shade was obtained, indicating that compound B is not a sterol but a triterpenoid. Compound B has no UV-absorption between 220–340 nm, and has main IR-absorption peaks (Nujol) at 3.05 (m), 9.6 (m), 10.3 (m) and 11.42 (S) microns (Figure 1, sample V-25-1)[5]. It is probably Taraxasterol[6].

(3) Compound C, m.p. 147–150 °C, $[\alpha]_D^{20}$ – 27.25, – 26.05 ($CHCl_3$). Analysis found: C, 84.06, 84.15; H, 11.54, 11.74; mol. wt. 374, 362. Calc. for $C_{29}H_{50}O$: C, 83.99; H, 12.15; mol. wt. 414.69. *Acetate*, m.p. 130–132 °C, $[\alpha]_D^{20}$ – 47.5 ($CHCl_3$). Analysis found: C, 81.39, 81.61; H, 10.86, 10.76; mol. wt. 385. *Benzoate*, m.p. 149–150 °C; $[\alpha]_D^{20}$ – 19.68 ($CHCl_3$). Analysis found: C, 83.59, 83.43; H, 10.17, 10.19; mol. wt., 443. Compound C has no UV-absorption between 220–340 nm. It has main IR-absorption peaks (Nujol) at 2.98 (S), 9.42 (S), 9.77 (m), 10.32 (m) and 12.5 (m) μ (Figure 2, sample V-24-1), gives a positive Liebermann-Burchard reaction (blue to green), and is probably γ-sitosterol[7].

The total steroidal and terpenoidal fraction has been found by Prasad[8] to have no significant antiinflammatory activity when tested by the carragenin method.

After petroleum ether extraction, *P. lanceolata* was extracted exhaustively with ethanol by cold percolation. The solvent free extract was extracted with dilute hydrochloric acid. The acidic extract was found to contain very little tertiary bases, as tested by Mayer's reagent and paper chromatography, but contained considerable amounts of water-soluble quaternary bases which were precipitated by ammonium reineckate. The base reineckates were dissolved in acetone, decomposed with silver

[1] V. N. Dwivedi, *Bhavaprakash Nighantu* (Hindi translation) (Motilal Banarsi Das, Banaras, India 1949), p. 52; R. N. Chopra, I. C. Chopra, K. K. Handa and L. D. Kapur, *Indigenous Drugs of India*, 2nd edn (U.N. Dhur and Sons Ltd., Calcutta, India 1958), p. 520; K. R. Kirtikar and B. D. Basu, *Indian Medicinal Plants* (L. M. Basu, Allahabad, India 1933), vol. 2, p. 1345.

[2] D. N. Prasad, K. D. Gode, P. S. Sinha and P. K. Das, Indian J. med. Res. *53*, 1062 (1965).

[3] D. N. Prasad, S. K. Bhattacharya and P. K. Das, Indian J. med. Res. *54*, 582 (1966).

[4] All microanalyses were carried out by Dr. G. Weiler and F. B. Strauss, Microanalytical Laboratory, Oxford, England.

[5] The author is indebted to Dr. M. N. Mitra, St. Louis, Missouri, USA for all UV- and IR-spectra carried out respectively in Cary's Spectrophotometer (in ethanol) and in Model 21 Double Beam Infra Red Spectrophotometer, Perkin Elmer Corporation, USA in Nujol mull (1X, 5X – ordinary and expanded Scales).

[6] E. H. Rodd, *Chemistry of Carbon Compounds* (Elsevier Publishing Company, Amsterdam – New York 1953), vol. 2, part B, p. 730.

[7] *Elsevier's Encyclopaedia of Organic Chemistry* (Elsevier Publishing Company, Inc. New York – Amsterdam 1940) 14S, 1803.

[8] D. N. Prasad, unpublished report.

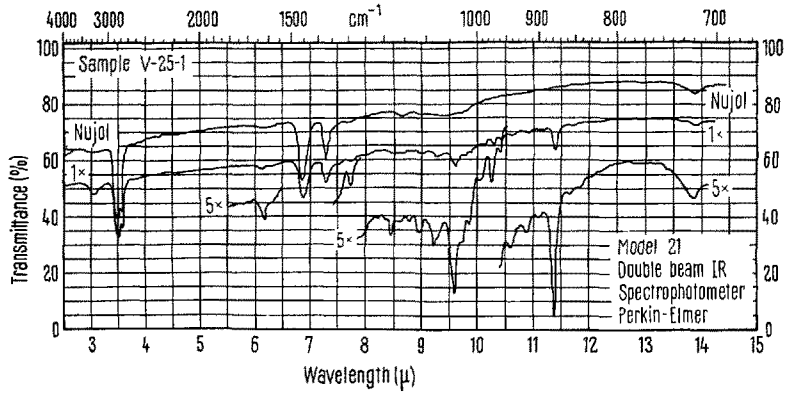

Fig. 1. IR-absorption curve (Nujol) of compound B (V-25-1). V-25-1 is a code number used to indicate a compound numbered 1 which has been entered in page 25 of the research book No. V.

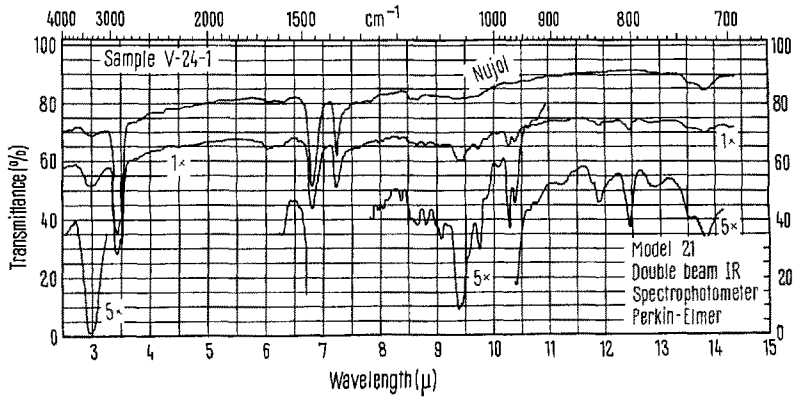

Fig. 2. IR-absorption curve (Nujol) of compound C (V-24-1).

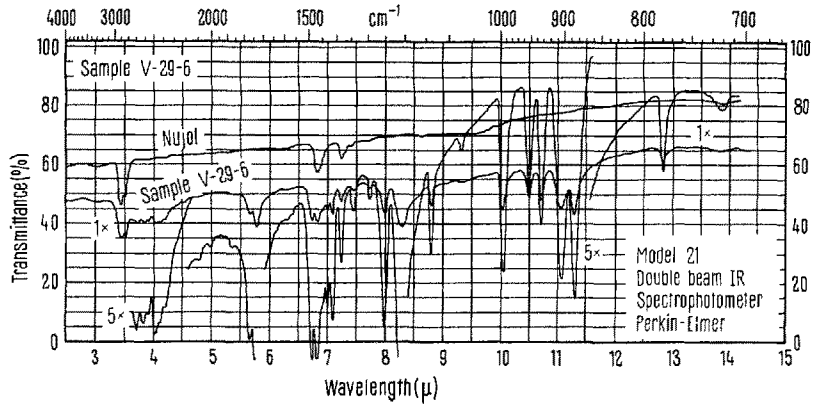

Fig. 3. IR-absorption curve (Nujol) of Pluchine (V-29-6).

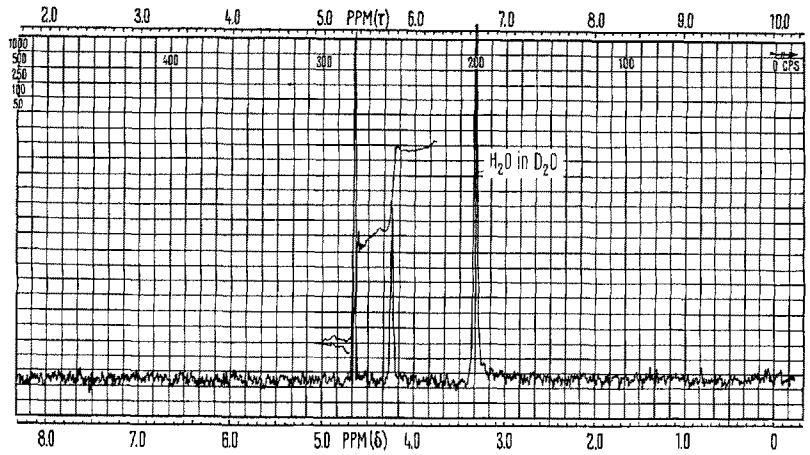

Fig. 4. NMR-spectrum of Pluchine in D_2O (V-33-2).

sulphate solution, and then converted into base chlorides with barium chloride. The residue obtained on evaporation of the solution to dryness was dissolved in absolute alcohol, and the alcoholic solution was passed through a column of aluminium oxide for chromatography. The first few fractions of the eluant yielded hygroscopic crystalline needles, identified as choline chloride by comparison with an authentic specimen. The later fractions of the alcoholic eluant yielded crystalline needles of a new quaternary base chloride named as Pluchine, m.p. 243–244 °C (decomposition and volatalization with evolution of gas), $[\alpha]_D^{20}$ – 29.51 (H_2O). Analysis found: C, 38.74, 38.59, 39.12, 39.09; H, 7.79, 7.67, 7.78, 7.81; N, 8.64, 8.30, 7.49, 7.68; cl, 23.55, 23.80, 22.85, 22.80. With alcoholic picric acid Pluchine gave a picrate, m.p. 181–182 °C. Analysis found: C, 38.46, 38.58; H, 3.87, 4.08; N, 16.09, 15.86; mol. wt. (Rast), 690, 674. Pluchine has no UV-absorption peaks between 220–340 nm, but has IR-absorption peaks (Nujol) at 3.75, 3.85, 3.95, 4.06, 4.17, 4.25, 4.8, 5.0, 5.36, 5.67, 5.8 (S), 6.75 (S), 7.05, 7.15 (S), 7.46, 7.75, 8.02 (S), 8.3 (S), 8.82 (S), 9.33, 10.1 (S), 10.54 (S), 10.75 (S), 11.1 (S), 11.35 (S) and 12.88 (S) μ (Figure 3, sample V-29-6). The NMR-spectrum[9] of Pluchine determined in D_2O solvent with TMS as external reference is given in Figure 4, sample V-33-2. When heated Pluchine decomposes with evolution of a volatile gas having amine-like smell and turning red litmus blue. The evolved gas was dissolved in alcohol and the alcoholic solution yielded with picric acid a picrate, needles, m.p. 219–222 °C, identified as trimethylamine picrate. Under identical conditions of heating acetylcholine chloride also yielded trimethylamine as the volatile component. As Pluchine has no UV-absorption, and as both Pluchine and acetylcholine chloride decompose on heating with evolution of trimethylamine, Pluchine has possibly an open chain structure with the trimethylammonium moiety at one end, similar to acetylcholine chloride.

On preliminary pharmacological investigations on rat's isolated intestine, SANYAL[10] found Pluchine to be a non-specific relaxant. It antagonises both acetylcholine and barium chloride induced spasms. It thus resembles Papaverine in its action. It was further observed by SANYAL[10] that Pluchine potentiates barbiturate-induced hypnosis in albino rats. Pluchine has also been found by PRASAD[8] to have antiinflammatory action in reducing carrageenin-induced inflammation in hind paw of albino rats when compared with cortisone taken as standard.

Thus, the cholinergic activity of the water-soluble portion of the alcoholic extract of *P. lanceolata*, as observed by PRASAD et al.[2] can be accounted for as due to the presence of choline in the drug. The smooth muscle relaxant spasmolytic action, central nervous system activity as evidenced by potentiation of barbiturate-induced hypnosis and the antiinflammatory activity of the water-soluble portion of the alcoholic extract as found by PRASAD et al.[2,3] are all due to the presence of a new quaternary base chloride, Pluchine, in *P. lanceolata*.

Further studies to elucidate the chemical structure of Pluchine are in progress. Detailed pharmacological investigations on Pluchine will be published shortly elsewhere.

Zusammenfassung. Es wird über die aktiven Prinzipien einer Pflanze, die in Indien zu Medizinalzwecken verwendet wird (*Pluchea lanceolata* I.), berichtet.

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[9] The author is indebted to Dr. J. MARKI, Varian AG, Switzerland, for determining the NMR-spectrum.

[10] A. K. SANYAL, unpublished report.

β-Ergokryptine, a New Alkaloid of the Ergotoxine Group[1]

Paper chromatographic analysis with the help of a special system (dimethyl phthalate impregnated paper as stationary phase, 20% formamide plus 80% citrate buffer pH 3.2 as mobile phase) has shown that certain ergokryptine preparations are not single substances but an isomorphically crystallizing mixture of 2 very closely related isomers which we would like to designate as α- and β-ergokryptine respectively. α-Ergokryptine corresponds to the alkaloid which at one time was described together with ergocristine and ergocornine as a component of the isomorphically crystallizing alkaloidal complex ergotoxine[2].

The separation of β-ergokryptine from α-ergokryptine in preparative quantities was achieved in the form of the salt with di-(*p*-toluyl)-L-tartaric acid. In ergokryptine preparations from Portuguese ergot an isomeric proportion α-: β-ergokryptine of 4:1 was established whilst Swiss cultivated ergot gave the proportion 2:1.

β-Ergokryptine $C_{32}H_{41}N_5O_5$ crystallized from benzene in rectangular plates m.p. 173° (decomp.) $[\alpha]_D^{20} = -174°$ (c = 1.5 in $CHCl_3$); – 91° (c = 2.0 in pyridine).

Epimerisation in alkaline or acid solution gave β-ergokryptinine $C_{32}H_{41}N_5O_5$. From methanol long needles, m.p. 220° (decomp.) $[\alpha]_D^{20} = +424°$ (c = 1.0 in $CHCl_3$); +492° (c = 1.0 in pyridine).

Catalytic hydrogenation of β-ergokryptine under the usual conditions for ergot alkaloids[3] led to 9,10-dihydro-β-ergokryptine, $C_{32}H_{43}N_5O_5$. From methanol or ethanol rhombic leaves, m.p. 194–195° (decomp.) $[\alpha]_D^{20} = -31°$ (c = 1.5 in pyridine).

On hydrolysis of β-ergokryptine under various conditions one equivalent of each D-lysergic acid, NH_3, dimethylpyruvic acid, rac. proline and L-isoleucine (natural *threo* form) were obtained. From these results and from the comparison of additional chemical, physical and spectroscopic data it follows that β-ergokryptine is differentiated from α-ergokryptine (earlier designated without

[1] 66. Mitteilung über Mutterkornalkaloide (65. Mitteilung: H. OTT, A. HOFMANN and A. J. FREY, J. Am. chem. Soc. *88*, 1251 (1966)).

[2] A. STOLL and A. HOFMANN, Helv. Chim. Acta *26*, 1570 (1943).

[3] A. STOLL and A. HOFMANN, Helv. Chim. Acta *26*, 2070 (1943).