evaporated from this solution and the oily residue was heated in an oil bath at 180—200° at a reduced pressure (10 mmHg) to effect degradation. The distillate was collected to 1.8 g (81.8%) of a colorless oil.

Hofmann Degradation of *cis*-Quinolizidine Methiodide (VII)——A solution of 2.2 g of VII dissolved in 60 ml of H_2O and added with 2.2 g of Ag_2O was stirred for 4 hr at room temperature, the reaction mixture was filtered, and the filtration residue was extracted with hot water. Water was evaporated from this extract solution under a reduced pressure and the residual oily substance was heated in an oil bath at 180—200° at a reduced pressure (10 mmHg) to effect degradation. This distillate of colorless oil was collected. Yield, 0.2 g (16.7%). Gas chromatographic analysis showed the product to be the same as that from the Hofmann degradation of *trans*-quinolizidine.

Des-N-methylquinolizidine Methiodide——A solution of 1 g of colorless oily des-N-methylquinolizidine (VIII and IX) dissolved in 10 ml of acetone and added 2 g of MeI was allowed to stand at room temperature for 12 hr, the crystals formed were collected, and recrystallized from EtOH to colorless crystals, mp 245—247°. Yield, 0.4 g (21.0%). *Anal.* Calcd. for $C_{11}H_{22}NI$: C, 44.78; H, 7.52; N, 4.75. Found: C, 44.84; H, 7.46; N, 4.54. Methopicrate, mp 149.5°.

Ether was added to the mother liquor left removal of the crystals of mp 245—247° and the crystals that precipitated out were collected and recrystallized to colorless crystals, mp 142—145°. Yield, 0.7 g (36.8%). *Anal.* Calcd. for $C_{11}H_{22}NI$: C, 44.78; H, 7.52; N, 4.75. Found: C, 44.70; H, 7.62; N, 4.68.

Reduction of Des-N-methylquinolizidine Methiodide (mp 142—145°)——A suspension of 1 g of des-N-methylquinolizidine methiodide (mp 142—145°) and AgCl freshly prepared from 2 g of $AgNO_3$ in *ca.* 50 ml of H_2O was stirred at room temperature for 4 hr. The mixture wsa filtered, the residue on the filter was washed with hot water, and the combined filtrate and washing was evaporated under a reduced pressure. The residual oily substance was dissolved in EtOH and submitted to catalytic reduction with 0.1 g of Adams PtO_2: The reduction mixture was filtered, 1 g of KI was added to the filtrate, and the mixture was reflexed on a water bath for 2 hr. The reaction mixture was filtered while hot, the filtrated, and ether was added to residual solution. The crystals that precipitated out were collected and recrystallized from acetone to colorless crystals, mp 160—163°, which showed no depression on admixture with 1-methyl-2-butylpiperidine methiodide synthesized by another route.

Dihydro-des-N-methylquinolizidine Methiodide——A solution of 2 g of des-N-methylquinolizidine, a colorless oily substance without being separation, dissolved in 100 ml of AcOH was submitted to catalytic reduction over 200 mg of 5% Rh-C. The catalytic mixture was filtered, AcOH was evaporated from the filtrate, and the residue was basified with H_2O and 10% NaOH. This alkaline solution was extracted with ether and the solvent was evaporated from the ether layer. The oily residue was dissolved in 10 ml of acetone, 4 g of MeI was added, and the mixture was allowed to stand for 12 hr. The crystals that precipitated out were collected and recrystallized from EtOH to colorless crystals, mp 242.5°. Yield, 0.4 g (10.3%). Methopicrate, mp 142.5°. Ether was added to the mother liquor left after separation of the crystals of mp 242.5° and the crystals that precipitated out were collected and recrystallized from EtOH–$(C_2H_5)_2O$ to colorless crystals, mp 160—163°. Yield, 1.8 g (46.2%).

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Studies on the Constituents of *Euphorbia ebracteolata* Hayata

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According to many old documents of Chinese orthodox medicine and Japanese phytologists, it is known that chinese antiphlogistic and skin disease drug, Lüju (閭茹) is originated

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from the root tuber of *Euphorbia ebracteolata* HAYATA or *E. adenochlora* MORR. et DECNE (Euphorbiaceae); the former drug is of a yellow color and when broken it discharges a yellow sap which after hardening becomes black like varnish. In that reason it was called "tsitou lüju (漆頭藺茹) (varnish head). The latter, called "tsáo lüju" (草藺茹) (herbaceous Lüju), is produced in central China as an inferior sort. It is white, but by heating it on iron the head becomes black.[2] The geographic distribution of *E. ebracteolata*, a perennial herb, is in the eastern and central parts of Japan and in Hokkaido. The underground part of the plant is corpulent and of a brownish yellow color; it exudes a deep yellow latex if cutted which does not become black after hardening. This fact and some others are at variance with the description given in the old documents as for the description of the plant from which the drug is originated; therefore it seems doubtful that this drug was obtained from the root tuber of *E.ebracteolata*. The constituents of this species had not been studied till now, therefore the authors started studying the root tuber as follows; the powder of root tuber was extracted with chloroform as illustrated in Fig. 1. Chloroform soluble fraction was condensed and chromatographed on silicagel column eluting with hexane and benzene.

From fraction A, colourless needles (I), mp 223—225°, were isolated and identified as β-amyrin acetate, and it was hydrolysed to β-amyrin, mp 189—190°.

Repeated recrystallization of fraction B from ethanol, gave colourless needles (II), mp 118—120°, which were found by GLC to be a pure substance and identified as 24-methylene-cycloartanol.[3] By acetylation it gave acetate as colourless needles, mp 110—112°.

Colourless needles (III), mp 138—139°, were obtained by recrystallization of fraction D from methanol, and the substance was identified as β-sitosterol, however, it was shown by GLC that it contained small amounts of campesterol and stigmasterol.[4]

From the methanol soluble fraction, sucrose was obtained as colourless crystals by recrystallization from methanol.

Fraction C was chromatographed on silicagel treated with 0.5M oxalic acid eluting with benzene.

From benzene eluate were obtained yellow needles, mp 144—145°, which became blue with ethylenediamine, reddish violet with 1,2-dinitrobenzene, reddish violet with methanolic lead acetate and reddish violet with nickel acetate. Infrared (IR) spectrum (KBr) showed absorption bands at 1663, 1633, 875 cm^{-1}. Ultraviolet (UV) spectrum showed a maximum at 322 mμ (1.8×10^4). Nuclear magnetic resonance (NMR) spectrum showed a singlet at

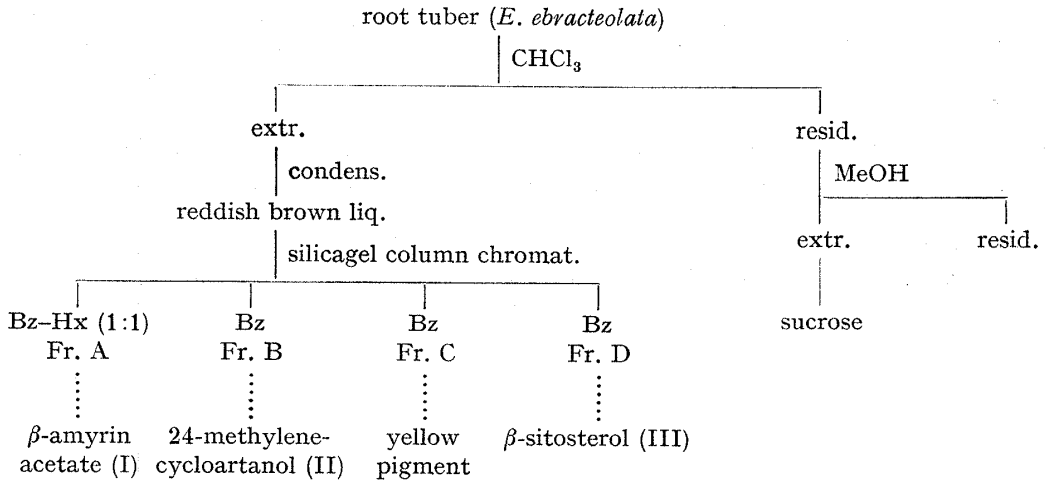

Fig. 1. Extraction and Purification of the Components

2) E. Bretschneider, *Botanicon Sinicum*, 3, 239 (1895).
3) G. Ohta, *Chem. Pharm. Bull.* (Tokyo), **8**, 5 (1960); *idem*, *ibid.*, **8**, 9 (1960).
4) T. Murakami, H. Itokawa, A. Matsushima, and N. Ikekawa, *Yakugaku Zasshi*, **83**, 4 (1963).

7.05 ppm (1H) and a doublet at 6.55 (2H) attributable to the aromatic protons, and a quartet due to a vinyl proton $\left(\underline{H}>=<\begin{matrix}H\\H\end{matrix}\right)$ at 5.95 (1H) and vinyl protons $\left(H>=<\begin{matrix}H\\\underline{H}\end{matrix}\right)$ at 5.05 (2H) respectively. The mass spectrum of this compound showed a peak at *m/e* 298 for the molecular ion. The authors will report later on this substance.

Experimental

Extraction——The powder of root tuber (500 g) of *Euphorbia ebracteolata* HAYATA was extracted with 3 liter $CHCl_3$ for 20 hr on the steam-bath. After filtration, the chloroform solution was concentrated *in vacuo* to a reddish brown syrup. This syrup was chromatographed on silicagel column. Fraction A was eluted with hexane and benzene (1:1). Successively fraction B, C, and D were obtained by eluting with benzene. After extraction with $CHCl_3$, the residue was extracted with methanol. After filtration, the methanol solution was condensed to a brown syrup (Fig. 1).

Isolation of β-Amyrin Acetate (I)——Fraction A was chromatographed on silicagel column. Elution with hexane and benzene (1:1) yielded a crystalline mass which was crystallized from acetone to give colourless needles, mp 223—225°. Liebermann-Burchard reaction positive. IR (CCl_4) cm^{-1}: 1739, 1256 (-$OCOCH_3$), 813 $\left(H>=<\right)$. NMR ppm: 5.18 $\left(1H, t, H>=<\right)$, 4.50(1H, m, proton at C_3), 2.03 (3H, s, CH_3COO-), identified as β-amyrin acetate by mixed melting point and comparison of IR and NMR spectra. β-Amyrin acetate was then hydrolysed to β-amyrin, colourless needles, mp 189—190°, identified with authentic sample by mixed melting point.

Isolation of 24-Methylenecycloartanol (II)——It was proved that fraction B contains three components, by testing it with thin-layer chromatography (TLC) treated with silver nitrate, and with gas liquid chromatography (GLC). Repeated recrystallization of fraction B from ethanol yielded colourless needles which were pure on gas chromatogram, mp 118—120°. IR (KBr) cm^{-1}: 3065, 1646, 887 (terminal methylene), 3026 (cyclopropane). NMR ppm: 4.77, 4.73 (2H, d, terminal methylene), 3.33 (1H, m, H–$\overset{|}{\underset{|}{C}}$–OH), 1.35 (6H, d, $J=16$ cps, isopropyl group), 1.07, 1.00, 0.98, 0.91, 0.83 (5-CH_3, s), 0.95 (CH_3, d, $J=5$ cps), 0.57 (1H, d), 0.33 (1H, d) cyclopropane ring. Mass Spectrum: 440 (M^+). This one was identified as 24-methylenecycloartanol by mixed melting point and comparison of IR and Mass spectra. Acetylation of (II) with Ac_2O and pyridine in the usual manner gave the acetate as colourless needles, mp 110—112°.

Identification of Campesterol, Stigmasterol, and β-Sitosterol——Repeated recrystallization of fraction D from methanol yielded colourless leaflets, mp 138—139°. IR (KBr) cm^{-1}: 2905, 1465, 1385, 1063, 1053, 959. NMR ppm: 5.03 (trisubstituted double bond), 3.45 (1H, m, H–$\overset{|}{\underset{|}{C}}$–OH). By GLC it was observed that this substance contained mainly β-sitosterol and two minor components, campesterol and stigmasterol.

Isolation of Sucrose——The brown syrup of methanol extract gave a precipitate after allowing it to stand at room temperature. After the recrystallization from methanol, the precipitate yielded colourless crystals, mp 174—175°, which were identified as sucrose by mixed melting point and comparison of IR.

Isolation of Yellow Pigment——Fraction C was chromatographed on silicagel column treated with 0.5M oxalic acid. Elution with benzene yielded yellow needles, which were recrystallized from benzene mp 144—145°. IR (KBr) cm^{-1}: 1663, 1633, (quinone), 875 (vinyl). UV λ_{max}^{EtOH} $m\mu$ (ε): 232 (8.6×10^3), 322 (1.8×10^4), 420 (2.6×10^3). NMR ppm: 7.05 (1H, s), 6.55 (2H, d), 5.95$\left(1H, q, \underline{H}>=<\begin{matrix}H\\H\end{matrix}\right)$, 5.05$\left(2H, q, H>=<\begin{matrix}H\\\underline{H}\end{matrix}\right)$, 2.85 (1H, q), 2.15 (-$CH_3$, s), 1.13 (-$CH_3$, s), 1.03 (-$CH_3$, s). Mass Spectrum: 298 (M^+).

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