dehydrating agents resulted in a mixture of (A) and (B). On periodic acid oxidation (A) afforded formaldehyde and vanillin.

Results and discussion. The compound (A), $C_{18}H_{18}O_6$ (on the basis of integral proton count and by difference of the sugar component from the parent compound, $C_{24}H_{28}O_{11} + H_2O - C_6H_{12}O_6$), showed significant IR absorption bands at 1700 cm^{-1} (α,β-unsaturated ester carbonyl) and at 1650 cm^{-1} (due to dissolved water in polyhydroxy system)[7]. It did not show the molecular ion peak in its mass spectrum, but instead intense fragment ions appeared at m/e 168 (fragmentation at 'a' and 'b'), 153 (m/e 168-Me), 131 (fragmentation at 'b') and 31 ($CH_2\overset{+}{O}H$); consistent with structure (II) for (A).

In contrast to a sharp one band at 1700 cm^{-1} in the IR-spectra of kutkin and component (A), the component (B), $C_{18}H_{16}O_5$ (M^+, m/e 312), showed a twin peak at 1710 cm^{-1} (CHO) and 1700 cm^{-1} (α,β-unsaturated ester). Again, the band at 1650 cm^{-1} ascribed to dissolved water in polyhydroxy systems[7], is completely absent in (B). The location of the aldehyde function in (B), associated with a OCH–CH= grouping, was confirmed from its NMR-spectrum, which exhibited a doublet at 9.8 δ (J = 4 cps). This evidence together with the mass spectral fragmentation pattern, significant peaks at m/e 164 (fragmentation at 'b'), 153 and 151 (fragmentation at 'a' and 'a[1]'), and 149 (m/e 164-Me), indicate structure (III) for (B).

On the basis of the structures (II and III) for the major degradation products of kutkin, we propose the revised structure (IV) for the glucoside. The physical data (NMR-[8] and IR-spectra, high optical rotation, $[\alpha]_D^{41}$ — 165°, and analysis. Found: C, 54.54, 54.81, 54.65; H, 5.55, 5.86, 5.81. Calc. for $C_{24}H_{28}O_{11}$, $2H_2O$ (IV): C, 54.54; H, 6.06. RASTOGI's formula, $C_{23}H_{24}O_{10} \cdot 2H_2O$ (I) requires: C, 55.6; H, 5.6. Found[1]: C, 55.0; H, 5.3) are also in agreement with the structure (IV) for kutkin[9].

Zusammenfassung. Neuer Strukturvorschlag für kutkin, den Inhaltstoff einer indischen Heilpflanze *Picrorhiza kurroa* Royle ex Benth.

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[8] Dr. U. SCHEIDEGGER, VARIAN AG, Research Laboratory, Switzerland, also opined, on the basis of NMR-data, for the above structural assignments of kutkin and its 2 major degradation products.

[9] Acknowledgments. The authors are grateful to Dr. U. SCHEIDEGGER, VARIAN AG, Research Laboratory, Switzerland for the NMR-spectra.

Distribution of Tetrahydrocannabinolic Acid in Fresh Wild Cannabis

Tetrahydrocannabinolic acid (THCA) was reported as a major active component in the wild, fresh cannabis plants grown in Sapporo district, Japan[1]. The active substances have been considered to be in the highest concentration at the flower of female plant. There have been, however, few surveys on the quantities of THCA with relation to the seasonal distribution at the different sections of the plant body. The object of this paper is to demonstrate that THCA seems to be contained in the parts in prosperous growth, and especially concentrated at the bractlet in the period when the seeds are at the peak of ripening.

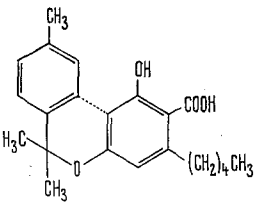
Materials and methods. Fresh, wild cannabis plants grown in Sapporo district, Japan, were collected for 5 months, from June to October. The amounts of THCA in top and middle leaves, bract, bractlet, flower, and flower bud were measured by using gas chromatography. As preliminary treatment, the samples dried at room temperature should be heated at 110 °C for 15 min in order to transfer THCA to tetrahydrocannabinol (THC) for the convenience of determination.

Analysis of bractlet. Fresh samples (2–4 mg), dried at room temperature for 1 or 2 days, were placed in small test-tubes which were then heated at 110 °C for 15 min in an electric regulative drying apparatus. After cooling, 0.1 ml of 0.5% ethanolic solution of tetramethyldiaminodiphenylmethane as an internal standard was added at 15 °C, with which the samples were extracted simultaneously for 20 min at room temperature. 1 or 2 µl of the THC-solution thus obtained was submitted to gas chromatography as described below.

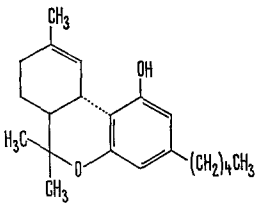
Analysis of leaf. Pulverized leaves (20–30 mg), dried at room temperature for 1 or 2 days, were heated as described above and were extracted 3 times with each 10 ml of methylene chloride at room temperature. The extract was submitted to column chromatography on silica gel (1 × 3 cm) using methylene chloride as the eluting solvent. The first 20 ml of eluate was evaporated in a small test-tube and the residue was dissolved in 0.1 ml of 0.5% ethanolic solution of the internal standard. 1 or 2 µl of the solution was then submitted to gas chromatography.

Gas chromatography. Run at 210 °C, using a stainless steel column (1 m × 4 mm) of 5% SE-52 on Shimalite W with a He flow of 30 ml/min and a hydrogen flame ionization detector.

Results and conclusion. Most of THC thus determined is present originally in the form of phenolic carboxylic acid, THCA, in the fresh plant and can readily be generated through the decarboxylation of the acid by heating or



Cannabinolic acid



Tetrahydrocannabinol

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Table I. Contents of tetrahydrocannabinolic acid in fresh samples at different periods

Date of collection (1968)	Sex	Top leaf of stem (%)[a]	Leaf of middle height of stem (%)	Bractlet (%)	Bract (%)	Flower (%)	Flower bud (%)
July, 17	Male	0.65	–	–	2.59	0.45	2.11
June, 19		1.47	0.61	–	–	–	–
July, 17	Female	1.86	1.20	–	4.77	–	–
August, 23		0.21	0.50	1.44	–	2.17	–
September, 17		0.12	0.19	5.62	–	–	–

[a] The % values are relative to the air-dried tissue weight and the means of 3 or 4 determinations with the relative mean deviation of ± 5%.

Table II. Contents of tetrahydrocannabinolic acid in bractlets at different periods

Date of collection (1968)	IX/1	IX/21	IX/28	X/9	X/19	X/30[b]
Contents (%)[a]	2.68	5.84	6.60	8.45	9.40	10.90

[a] The % values are relative to the air-dried tissue weight and the means of 3 or 4 determinations with the relative mean deviation of ± 5%. [b] The plant bodies were at the onset of withering.

during storage[2]. Since THCA can hardly be extracted with the ordinary organic solvents, the process of decarboxylation of the acid to THC as a preliminary treatment of analysis was essential for the measurement; without the treatment the values of THC obtained from fresh plants were very small. Although the dehydrogenation of THC to cannabinol on smoking was observed to some extent[3–5], the heating process at 110 °C for 15 min was unaffected for such transformation.

The amounts of THCA were thus found to be differently distributed in the various sections of the plant body at the different seasons and to be higher in the parts in prosperous growth. As shown in Table I, THCA, though determined as a figure of THC, was contained rather highly at the top leaf of stem in younger female plant and at the bract and flower bud in male plant. The remarkable increase of this compound in the bractlet was observed in proportion to the ripening of seeds and was the highest at the end of growth as shown in Table II, contrary to the rapid decrease in the top leaf.

Zusammenfassung. In Abhängigkeit der Entwicklungszeit wird in Wildhanfpflanzen die Verteilung des Tetrahydrocannabinols gaschromatographisch bestimmt.

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Zur Biosynthese der C_{9-10}-Einheit der Indolalkaloide

Es ist bekannt, dass die monoterpenoiden Indolalkaloide aus Tryptamin und Secologanin aufgebaut werden[1,2] und die einzelnen Alkaloidtypen (Corynanthe-, Aspidosperma-, Ibogatyp) durch nachfolgende Umlagerungen entstehen. Mevalonat, Geraniol bzw. Nerol werden spezifisch in das C_{9-10}-Fragment inkorporiert. Dagegen ist bisher ungeklärt, auf welche Weise der C_5-Körper bei den Indolalkaloiden entsteht. Ein spezifischer Einbau von Acetat konnte bislang nicht beobachtet werden[3–6]. Kürzlich wurde gezeigt[7,8], dass Glycin-(2-^{14}C) spezifisch in die C_{9-10}-Einheit des Ipecacuanha-Alkaloids Cephaelin eingebaut wird. Kuhn-Roth-Oxidation und nachfolgender Schmidt-Abbau ergaben, dass 15–18% der Radioaktivität im C-15 lokalisiert waren. Bei einer Applikationszeit von 10 Tagen wurden wesentlich höhere Einbauraten erzielt als bei 21tägiger Fütterungsdauer. Acetat-(2-^{14}C) ergab eine geringere Einbaurate in das Alkaloid als Glycin. In diesem Fall war die Radioaktivität gleichmässig zwischen C-14 und C-15 verschmiert. Acetat-(2-^{14}C) wurde aber in spezifischer Weise, entsprechend der Isopren-Regel, in β-Sitosterol eingebaut. Die Autoren schliessen daraus, dass zwei verschiedene Monoterpen-Einheiten in der gleichen Pflanze (*Cephaelis acuminata*) aus unterschiedlichen C_2-Vorstufen aufgebaut werden. Markiertes Leucin sowie Glycin-(1-^{14}C) wurden praktisch nicht in Cephaelin inkorporiert. GARG und GEAR[9] verfütterten Glycin-(2-^{14}C) ebenfalls an *Rauwolfia serpentina*-Pflanzen und unterwarfen Ajmalin und Reserpin einem chemischen Abbau. In der Reserpsäure und der 3,4,5-Trimethoxybenzoesäure waren 83% bzw. 15,5% der Radioaktivität lokalisiert. Kuhn-Roth-Oxidation und nachfolgender Schmidt-Abbau zeigten, dass das Kohlenstoffatom C-18 vom Ajmalin 15,5% der Radioaktivität enthielt. Die Autoren postulieren, dass Glycin als spezifische Vorstufe für das C_{9-10}-Fragment von Alkaloiden anzusehen ist.

Wir haben ebenfalls versucht, dieses Problem zu klären und verschiedene markierte Vorstufen an Sprosse von *Catharanthus roseus* appliziert. Die Fütterungsdauer be-