The proposed structure was also confirmed by mass spectroscopy[5]. The molecular ion peak of elsholtzidiol was obtained at m/e 184 and other characteristic peaks at m/e 169, 166, 151, 95, 89, 71 and 65 support the proposed structure (Figure 3). That one of the hydroxy groups is secondary and the other tertiary, was further confirmed by obtaining only a monoacetate of elsholtzidiol. The acetate (n_D^{32} 1.4682) on elemental analysis was found to contain C, 63.67; H, 8.02% (required for $C_{12}H_{18}O_4$ C, 63.72; H, 7.96%). The IR gave bands at 3365 cm^{-1} (OH group) and 1730 and 1247 cm^{-1} (CH_3–CO–OR)[6].

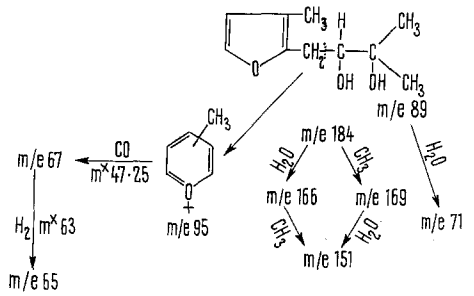


Fig. 3. Mass spectrum of Esholtzidiol.

Zusammenfassung. Isolierung und Strukturaufklärung von Esholtzidiol, einem Pflanzeninhaltsstoff aus *Esholtzia densa* Benth.

V. N. VASHIST and C. K. ATAL

Regional Research Laboratory, Jammu (India), 2 February 1970.

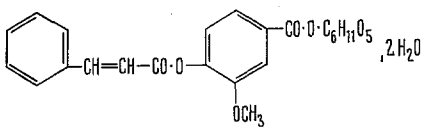
[5] K. HEYNS, R. STUTE and H. SCHARMANN, Tetrahedron *22*, 2223 (1966).

[6] We wish to express our gratitude to Dr. V. S. GUPTA of University of Saskatchewan for elemental analysis, NMR- and IR-spectra; Dr. D. H. G. CROUT of University of Exeter for mass spectrum and Dr. K. GANAPATHI, Director, Regional Research Laboratory, Jammu for his keen interest in this investigation.

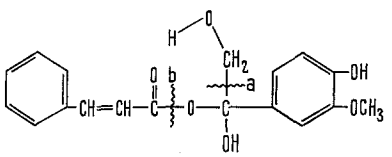
Chemistry of Kutkin, Isolated from *Picrorhiza kurroa* Royle ex Benth

RASTOGI et al.[1,2] previously isolated from the roots of *Picrorhiza kurroa* Royle ex Benth (Scrophulariaceae)[3] a bitter glucoside, kutkin, $C_{23}H_{24}O_{10}$, $2H_2O$, mp 211°, $[\alpha]_D^{41}$ — 165°, together with D-mannitol, vanillic acid, and several uncharacterized products. Kutkin, on hydrolysis, yielded vanillic acid, cinnamic acid and glucose, on the basis of which they put forward structure (I) for kutkin. In view of the uses of the drug reported in the indigenous and modern systems of medicine[4,5], we became interested in the chemistry of kutkin which appeared to be the active principle of the drug. Moreover, the structure (I) proposed for kutkin by RASTOGI et al. is not consistent with the biogenetic principles applicable to lignins[6], known to be derived from C_6 to C_3 and D-glucose precursors. Again, the facile hydrolysis of kutkin to glucose and other fragments in protic solvents, even at ordinary temperatures, also militates against the assumption[1] that the phenolic and sugar entities are joined in an ester linkage as shown in (I).

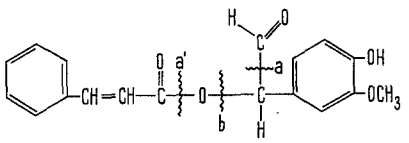
Materials and methods. The acid hydrolysate of kutkin, obtained by treating it with dilute hydrochloric acid, at room temperature, exhibited on papergrams 2 spots at Rf, 0.77 and 0.87, due to 2 reducing entities, besides the one at Rf, 0.26, due to glucose. The glucoside itself did not contain any reducing function. These results indicate that during the liberation of glucose from kutkin under mild acid treatment, the resultant aglycone develops 2 reducing entities, (A) and (B), having Rf, 0.77 and 0.87, respectively. The ratio of (A) to (B) also varied (ranging from 70 to 90) depending on the conditions of hydrolysis. The component (B) was isolated from the aqueous acidic solution by extraction with chloroform and from the mother liquor, (A) was later obtained by extraction with *iso*-amyl alcohol. Both the compounds on further hydrolysis, however, gave the same 2 products, viz. cinnamic and vanillic acids. Attempts to dry a sample of (A) over



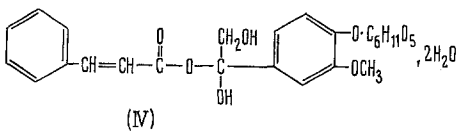
(I)



(II)



(III)



(IV)

One of us (K.B.) is indebted to the Ministry of Health, Govt. of India, New Delhi, for awarding a research fellowship. Financial assistance from the I.C.M.R., New Delhi, under the C.D.R.S. is gratefully acknowledged.

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[2] R. P. RASTOGI and M. L. DHAR, J. Sci. Ind. Res. *18B*, 219 (1959).

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[6] W. J. SCHUBERT, *Lignin Biochemistry* (Academic Press, New York 1965), p. 54.

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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Department of Medicinal Chemistry, Post Graduate Institute of Indian Medicine, and Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi-5 (India),
3 February 1970.

[7] G. EGLINTON, in *Physical Methods in Organic Chemistry* (Ed. J. C. P. SCHWARZ; Oliver and Boyd, Edinburgh and England 1964), p. 106.

[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.

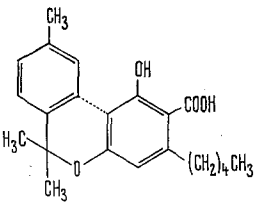
Materials and methods. Fresh, wild cannabis plants grown in Sapporo district, Japan, were collected for 5 months, from June to October. The amounts of THCA in top and middle leaves, bract, bractlet, flower, and flower bud were measured by using gas chromatography. As preliminary treatment, the samples 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.

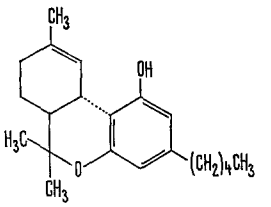
Analysis of leaf. Pulverized leaves (20–30 mg), dried at room temperature for 1 or 2 days, were heated as described above and were extracted 3 times with each 10 ml of methylene chloride at room temperature. The extract was submitted to column chromatography on silica gel (1 × 3 cm) using methylene chloride 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

[1] K. OKAMOTO, Rep. natn. Res. Inst. Police Sci. *20*, 109 (1967).