(III)[9,10] according to a modification of a procedure reported earlier[5,6,8].

Reaction of the 2'-hydroxyisoflavone (III) with ethyl bromoacetate in the presence of potassium carbonate gave the 2'-phenoxyacetate derivative (IV, mp 180–181°, IR 1725, 1653 cm^{-1} (C=O) (Nujol), UV λ_{max}^{EtOH} nm (log ε); 237 (4.54), 297 (4.10). Found: C, 65.02; H, 4.70. $C_{23}H_{20}O_8$ requires: C, 65.09; H, 4.75%). Treatment of IV with dilute alkali gave isoelliptic acid (V, mp 216–217°, IR 3250 (OH), 1730, 1640 cm^{-1} (C=O) (Nujol), UV λ_{max}^{EtOH} nm (log ε); 233 (4.56), 278 (4.02), 340 (3.81). Found: C, 62.04; H, 4.83. $C_{20}H_{18}O_8$ requires: C, 62.17; H, 4.70%) (lit. mp 204°[2], mp 193–194°[9]). By intramolecular cyclization with acetic anhydride and anhydrous sodium acetate, the acid (V) afforded dehydroisoelliptone (VI, mp 286–288°, IR 1633 cm^{-1} (C=O) (Nujol), UV λ_{max}^{EtOH} nm (log ε); 237.5 (4.51), 273 (4.39), 307 (4.30). Found: C, 68.40; H, 4.09. $C_{20}H_{14}O_6$ requires: C, 68.57; H, 4.03%). According to the MIYANO and MATSUI's method[11], VI was reduced with sodium borohydride in dioxane and subsequently, without any purification, followed by Oppenauer oxidation to give (±)-isoelliptone (II, mp 215–216°, IR 1680 cm^{-1} (C=O) (Nujol), UV λ_{max}^{EtOH} nm (log ε); 236 (4.59), 254.5 (4.09), 276 (4.01), 300sh (3.80), 335 (3.55). NMR[12]: 3.78_s, 3.82_s (each 3H, CH_3O), 3.9–5.2_m (4H, C-6, 6_a, 12_a), 6.54_s (1H, C-4), 6.80_q (1H, J = 2.0, 1.0 Hz, C-4'), 6.83_s (1H, C-1), 7.11_{bd} (1H, J = 1.0 Hz, C-8), 7.61_d (1H, J = 2.0 Hz, C-5'), 8.32_s (1H, C-11). Found: C, 68.14; H, 4.73. $C_{20}H_{16}O_6$ requires: C, 68.18; H, 4.58%). The mass spectrum data of II[13] (m/e: 352, 192, 191, 177, 161, 160, 149, 134, 131, 121, 106) is identical with that of natural isoelliptone which was reported by REED and WILSON[4].

Zusammenfassung. Die Synthese von Isoellipton aus 2'-Hydroxy-4',5'-dimethoxyfurano[3",2":6,7]isoflavon wird beschrieben.

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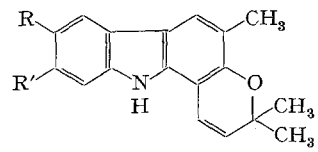
[12] The NMR-spectrum was measured with a Hitachi R-20 (60 MHz) spectrometer, using tetramethylsilane as the internal standard (δ-value in $CDCl_3$; *s*, singlet; *d*, doublet; *bd*, broad doublet; *q*, quartet).
[13] The mass spectrum measured by a Hitachi RMU-6D Mass Spectrometer.

New Alkaloids from *Murraya koenigii* Spreng.[1]

Current interest in the biosynthesis of alkaloids and the publication of 2 recent communications[2,3] prompt us to report our results on the structures of 2 of the new bases isolated by us from the leaves of *Murraya koenigii* Spreng.

The first new alkaloid, koenigicine, $C_{20}H_{21}NO_3$ (mol. wt. 323 by mass spectrum), mp 224–225°, is optically inactive. Its UV-spectrum, $\lambda_{max.}^{EtOH}$ 239, 300, and 361 nm (log ε 4.56, 4.52, and 3.97 respectively) indicated the presence of a carbazole nucleus already established in its congeners. This was supported by its IR-spectrum which showed peaks at 3420 (–NH), 1635, 1620, and 1600 cm^{-1} (unsaturation and aromatic system). The NMR-spectrum[4] showed the following signals (τ, multiplicity, number of protons under the peak, and assignment given): 8.5, singlet, 6, $-O-C(CH_3)_2$; 7.67, singlet, 3, $\geqslant C-CH_3$; 6.14 and 6.04, both singlets, 3 each, 2 methoxyls attached to an aromatic ring; 4.37 and 3.42, both doublets (J = 10 cps), 1 each, olefinic protons. In addition, the spectrum showed 3 aromatic protons – all singlets at τ 3.17, 2.60, and 2.50 respectively as well as a broad signal for a single proton, exchangeable with D_2O at 2.19 τ (>N–H). The mass-spectrum showed, apart from the M^+ peak at 323, abundant ions at m/e 308, 293, 292, 264, 250 and 154. Supporting evidence for the M^+ peak at 323 and for some of the important ions was provided by the appearance of doubly charged ions at 161.5, 146.5 and 132.5. All this evidence could be summarized in the structure (I):



(I) R = R' = OMe
(V) R = OMe; R' = H
(VI) R = H; R' = OMe

On catalytic hydrogenation over Raney Ni in ethanol, or over Pt in glacial acetic acid, koenigicine yielded the dihydro-derivative (II), $C_{20}H_{23}NO_3$, mp 232° (M^+ 325, by mass-spectrum); $\lambda_{max.}^{EtOH}$ 238, 267, and 325 nm (log ε 4.58, 4.25, and 4.21 respectively). The NMR-spectrum of this compound showed replacement of olefinic protons by

[1] Communicating No. 1353 from the Central Drug Research Institute, Lucknow.
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[4] All spectra were taken in $CDCl_3$ on a Varian A-60D machine using TMS as internal standard.

2 methylene groups at 8.14 and 7.22 τ (both triplets; $J = 7$ cps). Methylation with methyl iodide and sodium hydride in dry benzene gave N-methylkoenigicine (III), $C_{21}H_{23}NO_3$, mp 189°; $\lambda_{max.}^{EtOH}$ 241, 303, 346 and 361 nm (log ε 4.55, 4.56, 3.92 and 3.82 respectively), which on catalytic reduction (Pt-AcOH) yielded N-methyldihydrokoenigicine (IV), mp 193–194°, $C_{21}H_{25}NO_3$ (M^+ 339 by mass-spectrum); $\lambda_{max.}^{EtOH}$ 242, 273, and 318 nm (log ε 4.58, 4.28, and 4.22 respectively). The mass-spectrum of IV showed, apart from the M^+ peak, ions at m/e, 324, 283, 268, 240, 225, and 169.5 [m* 309.6 (339 → 324); 253.7 (283 → 268); and 236.2 (339 → 283)]. The combined data on II, III, and IV thus provide interlocking evidence for the structure of koenigicine as I.

The second base, $C_{19}H_{19}NO_2$, mp 194–195° (mol. wt. 293 by mass-spectrum) is again optically inactive and is shown to be identical with koenimbin recently isolated from the stembark of *Murraya koenigii*[3]. The following derivatives were prepared: dihydrokoenimbin, mp 249°; UV, $\lambda_{max.}^{EtOH}$ 243, 258, 312, 332, and 346 nm (log ε 4.21, 4.07, 4.03, 3.62 and 3.22), N-methylkoenimbin, mp 148°, $\lambda_{max.}^{EtOH}$ 242, 301, 339, 357 and 371 nm (log ε 4.55, 4.26, 3.83, 3.75 and 3.68 respectively), N-methyldihydrokoenimbin, mp 180°, $\lambda_{max.}^{EtOH}$ 247, 266, 314, 337 and 352 nm (log ε 4.49, 4.33, 4.29, 3.73 and 3.51 respectively). Our data is in agreement with the findings of NARASIMHAN et al.[3]. We think, however, that although the structure (V) proposed by the latter for koenimbin is highly likely to be correct, the alternative structure (VI) cannot yet be ruled out. This point can be settled only by synthesis, which is in hand.

These alkaloids are of considerable biogenetic interest. As an alternative to the suggestion of CHAKRABORTY and DAS[2] that the formation of the carbazole ring in plants precedes C-methylation of the aromatic ring by electrophilic attack, we propose that the biosynthesis of these alkaloids (and, in particular, the origin of the 3 or 6 C-methyl) involves the intermediacy of MVA. Appropriate tracer experiments on this aspect of the problem are in hand.

Zusammenfassung. Zwei neue Alkaloide, Koenigicine und Koenimbin, wurden aus Blättern von *Murraya koenigii* isoliert und als (I) und (V oder VI) charakterisiert. Es wird vermutet, dass bei der Biosynthese dieser Alkaloide das 3-C-Methyl aus Mevalonsäure stammt.

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Central Drug Research Institute, Lucknow (India), 24 March 1969.

Hepatic DNA Synthesis After Partial Hepatectomy in Rats Treated with Protamin-Zn-Insulin Under Different Nutritional Conditions

In the liver tissue remaining after 65–70% hepatectomy (PH) the changes suggesting the shift in the metabolic and endocrine balance develop regularly. Among these changes especially the rise of triglycerides content[1], the increase of fatty acids oxydation[2], the decrease of fatty acids synthesis[3] and the decrease of glycogen content[4] could be taken into account. The development of these changes is, as a rule, dependent on the stimulation of sympatho-adrenal system[5] and on the activation of the axis hypophysis-adrenal cortex[6]. The relationship of the changes in the metabolic and endocrine balance after PH to the liver regenerating process is not yet clear. In our experiments devoted to the study of this relationship, we also intended to determine the development of liver regeneration after PH in rats that received an s.c. injection of protamin-Zn-insulin (PZI) (Spofa, Czechoslovakia), the hormone influencing the metabolic and endocrine balance in the opposite way as compared with glucocorticoids and catecholamines.

Methods. For the experiments male rats (230–280 g), fed the standard laboratory diet[7] containing 25% of proteins, 53% of carbohydrates and 22% of lipids, were used. At partial hepatectomy 65–70% of liver tissue was removed[8]. The operations were performed at 10.00 h. One hour before death, all rats received an injection of thymidine-C^{14} (2.5 μc/100 g body wt.; specific activity, 44 mc/mM) into the femoral vein. The rats were killed by decapitation. The nuclei of the liver cells were isolated[9], then washed on filters with trichloroacetic acid (5%), alcohol and ether. The dry sample had been dissolved by hyamin before the scintillation fluid was added. The radioactivity of samples was measured in liquid scintillation counter Mark I (Nuclear, Chicago). The content of DNA in the liver tissue was estimated according to DISCHE[10]. The results were evaluated statistically using Student's t-test.

Results and discussion. In the first experiment, rats fed ad libitum before the operation and after it were used. PZI (3 IU/100 g body wt.) was injected 1 h before PH. In the same interval before the operation, the control rats received an injection of saline (0.1 ml/100 g body wt.). In rats that received insulin, the hepatic DNA synthesis 20 ($p < 0.01$) and 44 h after the operation ($p > 0.05$) was

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