In the *trans*-10-methyldecalin series, compounds which possess a chair-chair conformation, the axial methyl group is shifted downfield by 0.22 ppm when the carbonyl group is placed in the 2-position. This value is in good agreement with the value found in the steroid series and this result indicates that the steroid chemical shift data are applicable to the 10-methyl-decalin series. We have now also shown that the ORD curves for *cis*- and *trans*-10-methyl-2-decalones obtained in carbon tetrachloride solution were not significantly different from those found in methanol and dioxane solution.

In the *cis*-10-methyldecalin series, it was found (see Table) that introduction of a carbonyl group at the C-2 position shifted the C-10 methyl resonance downfield by 0.22–0.29 ppm. These values are to be compared with values of 0.11 ppm found in the coprostane series for A/B-*cis* compounds possessing the two chair conformations arranged in the steroidal form 1a. The decalin values found indicate that the spatial arrangement between the carbonyl and C-10 methyl group in the *cis*-10-methyl-2-decalones is similar to that found in the *non-steroidal* conformation 1b where the C-10 methyl group is axial to the ring holding the carbonyl group. Such a spatial arrangement has been assigned earlier on the basis of conformational analysis[1]. If the *twist* conformation 1c favored by ORD studies were the sole or major conformation, the angular methyl group would be diamagnetically shielded by the carbonyl group and would have its resonance band at about the same position as in the parent hydrocarbon[6].

It previously had been demonstrated by low temperature circular dichroism technique[3] that with the *cis*-7,7-dimethyl- and *cis*-7α-isopropyl-10-methyl-2-decalones one is dealing with a mixture of conformers. That a similar situation also exists for the parent *cis*-10-methyl-2-decalone has now been demonstrated by a study of the change of its NMR-spectrum with temperature. It was found that at − 20° to − 30° there was a band broadening and at − 65° to − 70° there was an increase in the fine structure of the spectrum.

Thus, the room temperature NMR-spectrum of the *cis*-10-methyl-2-decalones indicate that the *non-steroidal* conformation 1b is a major contributor to the conformational equilibrium. *The sign of the Cotton curve must be controlled by a conformer present in minor amounts and which has a large rotational value*[7]. Indeed, the twist conformation 1c could be such a minor contributor since it has been shown that such a conformation has a much higher Cotton effect magnitude than the standard chair form of cyclohexanones[8].

In the Table are also listed the chemical shifts of the related *cis*- and *trans*-10-methyl-4-decalones. Here again the chemical shifts of the two isomeric ketones relative to their parent hydrocarbons are about the same. As in the 2-decalone cases, it would appear that in the *cis* isomer the *non-steroidal* conformation predominates.

In all of the NMR-studies, however, it is realized that such a spectral investigation does not permit an unequivocal analysis of the conformations present. The temperature dependence of the CD studies does show that the change in free energy with temperature is greater for the lesser conformation (or conformations) which controls the sign of the ORD or CD curve. The entropy of the minor conformation (or conformations) must be larger than that of the major conformation and such a relationship would be expected between a twist and a chair conformation[9,10].

Zusammenfassung. Im Kernresonanzspektrum einiger substituierter *cis*-2-Decalone wurden die chemischen Verschiebungen angulärer Methylgruppen untersucht. Dabei wurde festgestellt, dass die Verbindungen vorwiegend in der nicht-steroidalen Sessel-Sessel-Konformation vorliegen. Das Vorzeichen der ORD Cotton-Kurven dieser Ketone muss durch das untergeordnete Vorhandensein einer Konformation sehr starker Amplitude, wie z. B. der Twist-Form, bedingt sein.

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[10] Added in proof: Since this paper was submitted, Elliott, Robinson, and Ridell[11], using the NMR-method, have arrived at similar conclusions.

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Alkaloid Studies LV[1]. 19-Dehydroyohimbine, a Novel Alkaloid from *Aspidosperma pyricollum*

The recent encounter[2] in various *Aspidosperma* species of congeners of the biogenetically intriguing alkaloid uleine (I)[3] prompted a reinvestigation – using more refined separation techniques coupled with mass spectrometry[4] – of the bark of *Aspidosperma pyricollum* Muell.-Arg. from which only uleine (I) had been isolated previously[5]. In the present study, aside from uleine (I), there was encountered (−)-apparicine (II)[6], a trace of demethyl-aspidospermine (III)[7], yohimbine (IV), β-yohimbine (V),

and a novel alkaloid, which in the sequel will be shown to possess the structure of 19-dehydroyohimbine (VI). The latter three substances occurred only in the strongly basic alkaloid fraction and represent the three principal alkaloids of this plant.
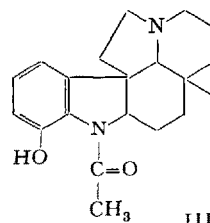
The new alkaloid, m.p. 245° (dec.), $[\alpha]_D^{27}$ + 106° (c, 0.53 in pyridine), exhibited an UV-absorption spectrum (λ_{max}^{EtOH} 226, 283, 293 mμ, log ε 4.48, 3.85, 3.77) very similar to that of yohimbine (IV) as well as IR-bands at 2.90 μ (NH) and 5.80 μ. The empirical formula $C_{21}H_{24}N_2O_3$ (Found: C 71.00, H 6.90, N 7.74, mol. weight 352 (mass spec.); calcd. C 71.57, H 6.86, N 7.95, mol. weight 352)
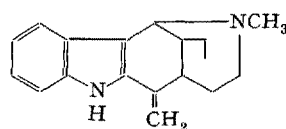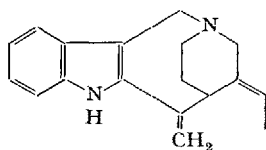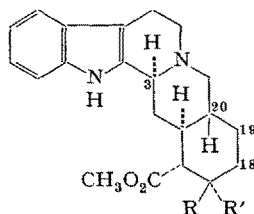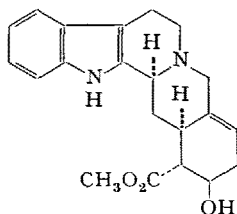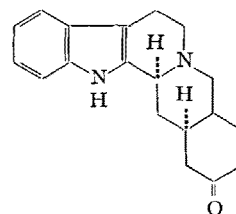
I   II   III

IV   R = OH;   R' = H
V   R = H;   R' = OH

VI   VII

indicated the presence of two less hydrogens than yohimbine. That this was due to a double bond rather than another ring was substantiated by the NMR spectrum (all spectra were run in $CDCl_3$ with TMS = $\delta 0.00$ ppm), which exhibited a signal at 5.55 ppm due to a single olefinic proton as well as resonances already observed with yohimbine due to NH (7.90 ppm), four aromatic protons (7.0–7.5 ppm), carbomethoxyl group (3.80 ppm) and the C-3 hydrogen atom (4.37 ppm).

The mass spectrum exhibited an intense molecular ion peak (m/e 352 of 100% relative intensity) as well as fragment ion peaks at M-1 (m/e 351, 93%), 184 (11%), 170 (39%), 169 (56%) and 156 (70%), all of which are typical[8] of yohimbine (IV) and its isomers. Noteworthy, however, are the great intensity differences, especially the weakness of the m/e 184 peak (a) and the abundance of the m/e 156 peak (b). If one attributes these differences from yohimbine to the presence of the trisubstituted double bond indicated by the NMR spectrum, then only its location between carbon atoms 19 and 20 will explain the inhibition of formation of ion a (unfavourable vinylic cleavage) and the increased production of ion b (favored allylic fission). These conclusions and the overall structure of the alkaloid were verified by the following transformations.
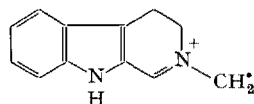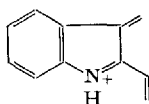
a, m/e 184   b, m/e 156

The presence of an axial hydroxyl group as in yohimbine (IV) was established by acetylation to an amorphous O-acetate (IR-absorption band at 8.0 μ, mass spectral molecular ion peak at m/e 394), which resulted in a downfield shift of the NMR signal associated with the hydrogen atom attached to the carbon bearing the hydroxyl group from 4.25 ppm in the alkaloid to 5.55 ppm in the acetate. Catalytic hydrogenation with platinum oxide in ethanol resulted in the uptake of one molar equivalent of hydrogen and the obtention of yohimbine (IV) as demonstrated by mixture melting point determination, IR and chromatographic comparison and rotation.

The alkaloid was very sensitive to oxidizing agents, including Oppenauer oxidations with potassium t-butoxide and fluorenone[9] as well as with aluminum phenoxide and cyclohexanone[10]. Oxidation could be effected

successfully with the MOFFATT reagent[11] in the absence of moisture or oxygen to yield the unstable, amorphous yellow β-keto ester (mass spectral molecular ion peak at m/e 350 corresponding to $C_{21}H_{22}N_2O_3$) which was completely enolic (λ_{max}^{CHCl} 5.85 and 6.03 μ; NMR signals corresponding to enolic hydroxyl proton at 12.3 ppm and single olefinic proton signal at 5.55 ppm) in contrast to the non-enolic dihydroanalog yohimbinone[9,12]. Decarbomethoxylation was effected by heating with $1 N$ hydrochloric acid[13] to give the α,β-unsaturated ketone VII, m.p. 226–228° (mass spectral molecular ion peak at m/e 292, λ_{max}^{CHCl} 5.98 μ). Migration of the double bond from the 19–20 to the 18–19 position was substantiated by the NMR spectrum of VII, which now exhibited two vinylic proton signals (doublets) centered at 5.92 and 6.65 ppm

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(J = 10 c/s). The alkaloid is thus unambiguously shown to be 19-dehydroyohimbine (VI) and to represent the third member of the rare class of 19-dehydroyohimbinoid alkaloids, the other two representatives being deserpideine[14] and raujemidine[15,16].

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Zusammenfassung. Ausser den bekannten Alkaloiden Ulein (I), Apparicin (II), Demethylaspidospermin (III), Yohimbin (IV) und β-Yohimbin (V) wurde noch ein unbekanntes Alkaloid aus der Rinde von *Aspidosperma pyricollum* Muell.-Arg. isoliert, dessen Struktur als 19-Dehydroyohimbin (VI) chemisch sowie spektroskopisch bewiesen wurde.

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Electron Microscopic Observations in the Substantia nigra of Mouse during Reserpine Administration

Distribution of catechol amines in the brain has been demonstrated histobiochemically by many investigators[1-4]. Catechol amine-containing cells in the Substantia nigra and neostriatum (putamen and caudate nucleus) were described by HILLARP et al.[5,6]. Recently WOOD and BARNETT[7] reported the appearance of catechol amine-containing granules in the ventromedial nucleus of the hypothalamus at a fine structural level. The storage of catechol amine in cytoplasmic granules in a bound state was reported by BERTLER, HILLARP, and ROSENGREN[8]; one can regard the storage as the final step in the formation of amines, making them available for physiological need. And it can be assumed that norepinephrine, 5-hydroxy-tryptamine, and dopamine are directly formed in the brain, judging from the relative impermeability of the blood-brain barrier to these amines[9].

Since reserpine blocks the storage of catechol amines in the brain[10], the present experiment was focused on further study of effects of reserpine on granules of brain monoamines, their morphological features and distribution at a fine structural level.

Materials and method. Twenty mice, six months of age, weighing an average of 30 g, were used in the present experiment. One group of animals was treated with reserpine (sedaraupin) 0.5–1 mg/kg i.p. daily. The animals showed clear-cut symptoms of sedation. 5 mice were sacrificed after 24 h, and 5 after one week for the purpose of electron microscopy. Another group of 10 untreated mice was used as controls.

The whole brain was removed as quickly as possible from the subjects, cut transversely through the Substantia nigra, and fixed in 1% OSO₄ fixative buffered with 0.14 ml veronal acetate (pH 7.4) containing 0.9 m of sucrose per ml for 4 h. The tissue was dehydrated with graded ethanol and propylene oxide; during dehydration, regions of the Substantia nigra were harvested in small pieces under the dissecting microscope. These were then embedded in Epon 812 according to the method of LUFT[11], polymerized for several days at graded temperatures, and sectioned with a LKB microtome. Sections were stained with uranyl acetate and examined in a Siemens Elmi 1.

Observations. Polymerized blocks were reoriented to include only the region of Substantia nigra under the phase microscope. The basis pedunculi was used as a reference in identifying the cells in Substantia nigra, when 10 to 20 μ sections were examined under the phase microscope.

Polygonal-shaped nigra cells ranging from 11 to 15 μ showed small ovoidal mitochondria distributed throughout the cytoplasm.

There were numerous vesicles with electron dense core and less dense periphery ranging from 0.1 to 0.3 μ in diameter at the periphery of cytoplasm (Figure 1), in the axoplasm and axonterminals (Figure 2). In control animals, not all the cells contain such granular vesicles in cytoplasm, which may indicate the existence of functionally different cells or the active metabolic use of amine-containing granules.

In addition to these granular vesicles, there were clear vesicles ranging from 0.08 to 0.1 μ in diameter seen evenly distributed in the axonterminals (Figure 3).

There were evenly distributed polysomes, and the prominent nucleus had an indentation in which presumably the cytoplasmic centre lies. The structure of nucleolus in these cells showed the characteristic presence of several vacuoles.

A notable aspect in these cells is the appearance of an oval osmiophilic body without membrane in the cyto-

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