Die Eigenschaften, der in dieser Arbeit neu beschriebenen Produkte sind in der Tabelle zusammengestellt. Über weitere Einzelheiten wird an einer andern Stelle ausführlich berichtet.

H. IMMER, M. LJ. MIHAILOVIĆ[11],
K. SCHAFFNER, D. ARIGONI und O. JEGER

Organisch-chemisches Laboratorium der Eidgenössischen Technischen Hochschule, Zürich, 24. Oktober 1960.

Summary

On treatment of 3α-hydroxy-5β-steroids with lead tetraacetate formation of 3α,9α-oxides is observed. The yields of this ring closure reaction decrease significantly in the order 11-keto, $\Delta^{9,11}$-ene and ring-C saturated compounds.

Alkaloid Studies XXV[1]. The Structures of the Aspidosperma Alkaloids Cylindrocarpine and Cylindrocarpidine

In connection with our investigations on alkaloids from Brazilian *Aspidosperma* species, there has been recorded in preliminary form[2] the isolation of four new alkaloids, cylindrocarpine, pyrifolidine, pyrifoline and refractine. We would now like to report the structure elucidation of cylindrocarpine, as well as the isolation and constitution of a fifth alkaloid of this series, cylindrocarpidine.

Cylindrocarpine (II). The earlier investigations[2] showed that cylindrocarpine corresponded to $C_{29-30}H_{32-34}N_2O_4$ and that the four oxygen atoms formed part of a carbomethoxy group and a N-cinnamoyl-7-methoxydihydroindole moiety. Considerable clarification was provided by nuclear magnetic resonance (NMR) studies[3]. First, the NMR spectra of a variety of indole and dihydroindole alkaloids were measured, among them aspidospermine (I)[4], eburnamonine[5], ibogamine[6], diaboline[7], coronaridine[8], and akuammicine[9], and these measurements showed clearly that such NMR spectra can often be used for purposes of 'finger printing'. The NMR spectrum (Fig. 1) of aspidospermine (I) – in addition to the characteristic peaks for the aromatic methoxyl function ($\delta = 3.89$), the N-acetyl group ($\delta = 2.20$), the C-5 ethyl substituent ($\delta = 0.67$ for strong line at CH_3 group) and the lone hydrogen atom attached to C-13 which is not split ($\delta = 2.20$) by any neighboring proton—shows a series of peaks from $\delta =$ 2.90 to 3.31 which appear to be quite characteristic of the aspidospermine skeleton. A multiplet consisting of four lines centered around $\delta = 4.50$ is assigned to the proton on C-2, which couples its spin to the two protons on C-3. These same features are also found in the NMR spectrum (Fig. 2) of cylindrocarpine (II), thus suggesting that the same type of skeleton is also present in this alkaloid. In addition to the expected differences associated with the N-acyl substituent (absence of strong $\delta = 2.20$ peak due to the acetyl group in I and appearance of lines at 397, 413, 455, and 471 cps—relative to tetramethylsilane at 60 mc—associated with the *trans*cinnamoyl system), the most significant conclusion is the absence of a C-ethyl group. A peak at $\delta = 2.48$ due to the hydrogen atom at C-13 is still present and is not split by any neighboring hydrogen; therefore, C-5 must also be substituted and this represents the most logical site for the carbomethoxy bearing fragment known[2] to be present in cylindrocarpine. The proximity of the carbonyl group to C-5 accounts for the shift of the C-13 proton peak from $\delta = 2.20$ in I to 2.48 in II. Another very important piece of information came from the NMR proton count[10] by electronic integration, which demonstrated quite clearly the presence of 34 protons and thus automatically established the empirical formula as $C_{30}H_{34}N_2O_4$ instead of the equally acceptable $C_{29}H_{32}N_2O_4$.

The empirical formula, the nuclear magnetic resonance data suggesting an aspidospermine (I) skeleton and the earlier adduced chemical information[2] are most plausibly combined in expression II and this was proved as follows. Reduction of cylindrocarpine (II) with lithium aluminum hydride afforded a separable mixture of the N-γ-phenylpropyl derivative VI (m. p. 49°C, no amide absorption in infrared)[11] and the non-crystalline dihydrocyclindrocarpol VII ($\lambda_{max}^{CHCl_3}$ 6.10 μ)[11]. Oxidation of the latter with chromium trioxide in acetic acid solution provided the aldehyde, dihydrocylindrocarpal (VIII) ($\lambda_{max}^{CHCl_3}$ 5.85 and 6.10 μ), which was purified by chromatography and reduced by the Wolff-Kishner procedure to the known deacetylaspidospermine (IX)[12].

Cylindrocarpidine (IV). This new alkaloid was isolated from the cylindrocarpine (II) mother liquors[2] and exhibited the following properties after recrystallization from aqueous ethanol: m. p. 118–118.5°C, $[\alpha]_D^{27}$ – 122°C (c, 0.72 in chloroform), λ_{max}^{EtOH} 218 (log ε 4.58), 255 (4.09) and inflection at 286–290 mμ (3.61–3.55), λ_{min}^{EtOH} 234 mμ (log ε 3.67); *Anal.* Calc. for $C_{23}H_{30}N_2O_4$: C, 69.32; H, 7.59; N, 7.03; O, 16.06; 2 OCH_3, 15.55. Found: C, 69.22; H, 7.53; N, 6.98; O, 16.32; OCH_3, 15.41. The NMR proton count confirmed the analysis (found: 30.2 ± 0·5), while the NMR spectrum (Fig. 3) was very similar to that

[1] Paper XXIV, T. NAKANO, C. DJERASSI, R. A. CORRAL, and O. O. ORAZI, J. org. Chem., in press.

[2] B. GILBERT, L. D. ANTONACCIO, A. A. P. G. ARCHER, and C. DJERASSI, Exper. *16*, 61 (1960).

[3] For details on measurement and recording of NMR results see C. DJERASSI, T. NAKANO, A. N. JAMES, L. H. ZALKOW, E. J. EISENBRAUN, and J. N. SHOOLERY, J. org. Chem., in press. For reasons pointed out in that paper, dimensionless chemical shift units are used, δint/$SiMe_4$ = cps/60 × 10^6 ppm (for a 60 megacycle instrument). For conversion to the Tiers τ scale [G.V.D. TIERS, J. phys. Chem. *62*, 1151 (1958)] the following simple relationship may be used: $\tau = 10 - \delta$ int/$SiMe_4$.

[4] J. F. D. MILLS and S. C. NYBURG, Tetrahedron Letters, No. 11, 1 (1959); J. chem. Soc. *1960*, 1458. For confirmatory chemical evidence see H. CONROY, P. R. BROOK, and Y. AMIEL, Tetrahedron Letters, No. 11, 4 (1959). – G. F. SMITH and J. T. WROBEL, J. chem. Soc. *1960*, 1463.

[5] M. F. BARTLETT and W. I. TAYLOR, Tetrahedron Letters, No. 20, 20 (1959).

[6] M. F. BARTLETT, D. F. DICKEL, and W. I. TAYLOR, J. Amer. chem. Soc. *80*, 126 (1958).

[7] A. R. BATTERSBY and H. F. HODSON, Proc. chem. Soc. *1959*, 126.

[8] M. GORMAN, N. NEUSS, N. J. CONE, and J. A. DEYRUP, J. Amer. chem. Soc. *82*, 1142 (1960).

[9] K. AGHORAMURTHY and R. ROBINSON, Tetrahedron *1*, 172 (1957). – G. F. SMITH and J. T. WROBEL, J. chem. Soc. *1960*, 792. – K. BERNAUER, W. ARNOLD, C. WEISSMANN, H. SCHMID, and P. KARRER, Helv. chim. Acta *43*, 717 (1960. – P. N. EDWARDS and G. F. SMITH, Proc. chem. Soc. *1960*, 215.

[10] Specifically, the proton count was performed by integrating the 10 aromatic and olefinic protons, the proton on C-2 and the 6 protons of the methoxyl groups as a group of 17 protons, thus giving 408.4 ± 4 (arbitrary units) as an average of 5 runs. This is corrected for the residual $CHCl_3$ in the $CDCl_3$. The total integral for 5 runs was 839.4 ± 6 but a blank run of the solvent showed that 25 units of integral came from solvent contamination. Thus, the total integral is 814.4. This gives the total number of protons as 814.4 ± 6/408.4 ± 4 × 17 = 33.9 ± 0.5 protons.

[11] All substances gave satisfactory elementary analyses.

[12] See B. WITKOP and J. B. PATRICK, J. Amer. chem. Soc. *76*, 5603 (1954).

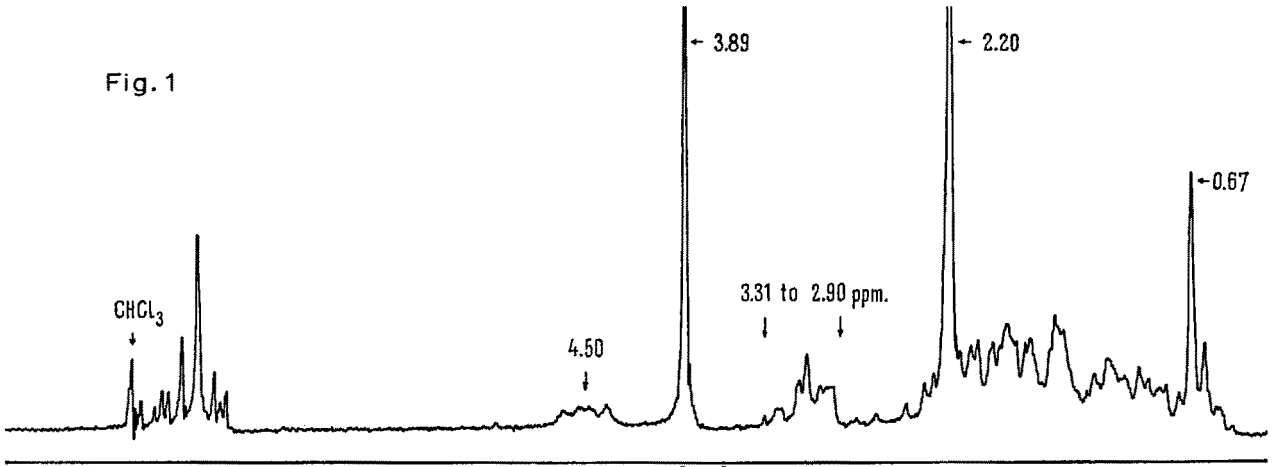


Fig. 1

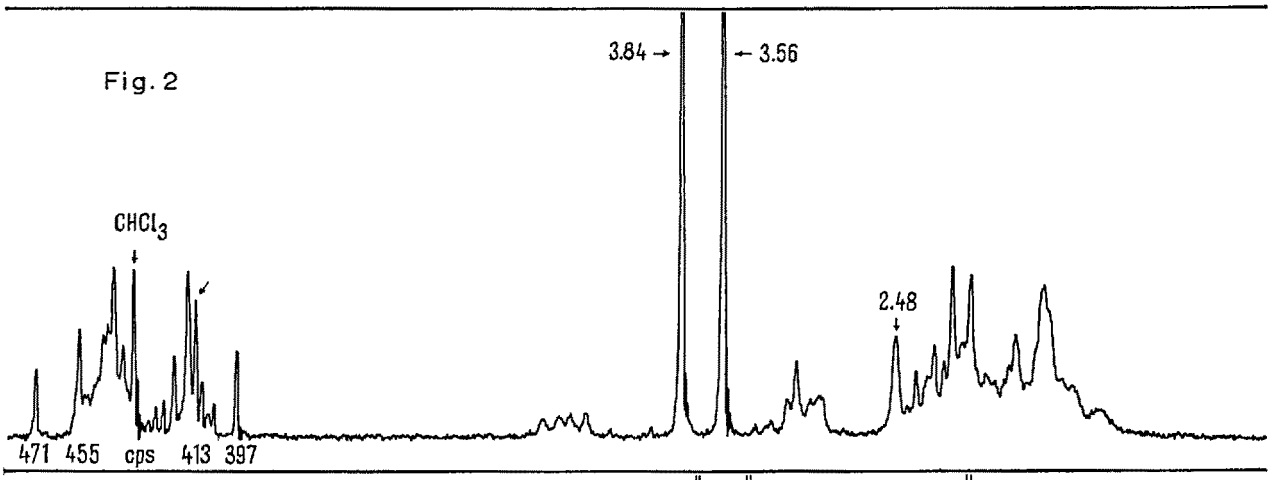


Fig. 2

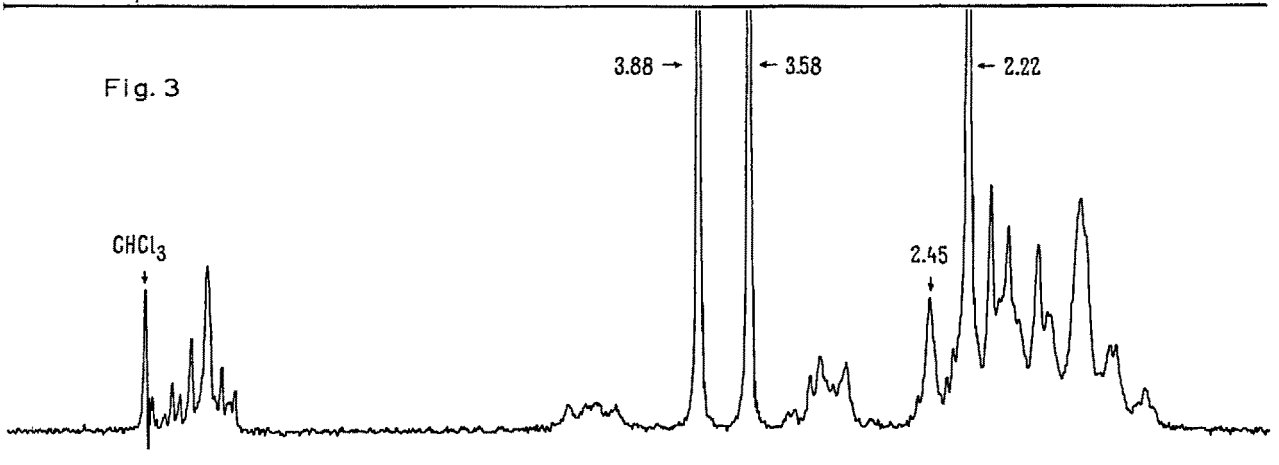
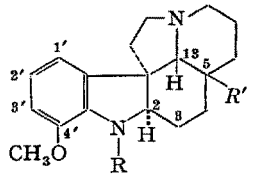


Fig. 3



I $R = COCH_3$; $R' = C_2H_5$
II $R = COCH{=}CHC_6H_5$; $R' = CH_2CO_2CH_3$
III $R = COCH_2CH_2C_6H_5$; $R' = CH_2CO_2CH_3$
IV $R = COCH_3$; $R' = CH_2CO_2CH_3$
V $R = H$; $R' = CH_2CO_2H$
VI $R = CH_2CH_2CH_2C_6H_5$; $R' = CH_2CH_2OH$
VII $R = COCH_2CH_2C_6H_5$; $R' = CH_2CH_2OH$
VIII $R = COCH_2CH_2C_6H_5$; $R' = CH_2CHO$
IX $R = H$; $R' = C_2H_5$

(Fig. 1) of aspidospermine (I) except for the absence of the C-ethyl group and the presence ($\delta = 3.58$) of a carbomethoxy function, which was also indicated by the infrared band at 5.81 μ (in addition to the amide band at 6.13 μ). The existence of a N-acetyl substituent ($\delta = 2.22$) was established by aqueous hydrochloric acid hydrolysis and paper chromatographic identification[13] of acetic acid in the steam-volatile distillate. An unsplit C-13 proton peak at $\delta = 2.45$ suggests that C-5 is again substituted in the manner of II. Indeed the non-volatile portion of this

[13] C. F. GARBERS, H. SCHMID, and P. KARRER, Helv. chim. Acta *37*, 1336 (1954).

[14] Postdoctorate research fellow, 1958–1960.

acid cleavage provided the dihydrochloride (m. p. 247 to 249°C) of cylindrocarpinic acid (V) [previously[2] obtained by acid hydrolysis of dihydrocylindrocarpine (III)], thus demonstrating that cylindrocarpidine had to possess structure IV.

Acknowledgement. Financial support has been provided by grant No. H-5048 of the National Heart Institute, National Institutes of Health (U. S. Public Health Service) and by the Conselho Nacional de Pesquisas, Brazil. The postdoctorate fellowship of B. G. in Rio de Janeiro was financed from a grant by the Rockefeller Foundation in support of a collaborative research program between Stanford University and the Instituto de Quimica Agricola, Rio de Janeiro.

C. DJERASSI, A. A. P. G. ARCHER[14], T. GEORGE, B. GILBERT[14], J. N. SHOOLERY, and L. F. JOHNSON

Department of Chemistry, Stanford University (Calif.), Instituto de Quimica Agricola, Rio de Janeiro (Brazil), and Varian Associates, Palo Alto (Calif.), July 29, 1960.

Zusammenfassung

Die Strukturaufklärung von Cylindrocarpin (II) und Cylindrocarpidin (IV) zeigt, dass diese zwei Alkaloide die ersten Mitglieder der Aspidosperminfamilie (I) sind, in denen eine sauerstoffhaltige Seitenkette anstatt der gewöhnlichen C-5-Äthylgruppe vorliegt.

Spontane Transsulfuration zwischen organischen Thiosulfonsäuren und Sulfinsäuren

Die organischen Thiosulfonate der allgemeinen Formel $R–SO_2–SH$ gewannen an biologischem Interesse, nachdem das Thiotaurin unter den Stoffwechselprodukten *in vivo* des Cystins[1,2] und der Alaninthiosulfonsäure, *in vitro* unter den Abbauprodukten des Cystins durch chemische[3] und enzymatische[4] Reaktionen nachgewiesen wurde.

Es schien uns nun von Interesse, zu erforschen, ob die Verbindungen der Formel $R–SO_2–SH$ spontan Erscheinungen von Transsulfuration aufweisen können, welche zu Verbindungen $R–SO_2–H$ führen.

Zu diesem Zweck wurden einerseits Alaninthiosulfonsäure (I) und Hypotaurin (II), anderseits Thiotaurin (IV) und Cysteinsulfinsäure (III) in 0,02 *M* wässeriger Lösung bei Zimmertemperatur gemischt und die Reaktionsprodukte alsbald papierchromatographisch untersucht (Whatman-Papier No. 4; Elution mit Phenol und Collidin-Lutidin).

Bei beiden Fällen waren im Chromatogramm alle vier erwähnten Verbindungen I–IV vorhanden, ausser dem geringe Spuren von Taurin, welches der Oxydation des Hypotaurins während der Chromatographie entstammte. Änderung des pH zwischen 5 und 8,5 sowie Verlängerung der Reaktionsdauer änderten den chromatographischen Befund nicht. Das Resultat zeigt, dass eine rasche Transsulfurationsreaktion zwischen den Verbindungen vom Typ $R–SO_2–SH$ und Akzeptoren vom Typ $R–SO_2–H$ stattfindet.

$$\underset{\text{I}}{R\text{SO}_2\text{SH}} + \underset{\text{II}}{R'\text{SO}_2\text{H}} \rightleftarrows \underset{\text{III}}{R\text{SO}_2\text{H}} + \underset{\text{IV}}{R'\text{SO}_2\text{SH}}$$

R = HOOC · CHNH$_2$ · CH$_2$– R' = NH$_2$ · CH$_2$ · CH$_2$–

Natürlich muss eine solche Transsulfurationsreaktion berücksichtigt werden, wenn biologische Materialien zwecks Feststellung von Thiosulfonaten untersucht werden sollen.

C. DE MARCO und M. COLETTA

Istituto di Chimica Biologica, Università di Roma (Italien), 25. Juli 1960.

Summary

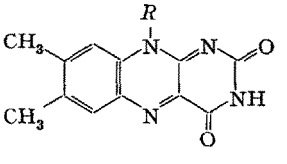
Spontaneous transulfuration reactions between alaninethiosulfonic acid and hypotaurine, and between thiotaurine and cysteinesulfinic acid, have been demonstrated.

[1] D. CAVALLINI, C. DE MARCO und B. MONDOVÍ, J. biol. Chem. *234*, 854 (1959).
[2] D. CAVALLINI, C. DE MARCO, B. MONDOVÍ und L. TENTORI, J. Chrom. *3*, 20 (1960).
[3] D. CAVALLINI, C. DE MARCO und B. MONDOVÍ, Arch. Biochem. Biophys. *87*, 281 (1960).
[4] D. CAVALLINI, C. DE MARCO, B. MONDOVÍ und G. MORI, Enzymologia, *22*, 161 (1960).

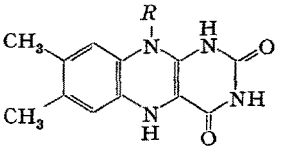
Über das Verhalten von Riboflavin-Semichinon gegen Metallionen. Modellstudien zur Metall-Flavoenzymkatalyse

Wir haben früher[1] gezeigt, dass Riboflavin, welches in der Chinonform Rfl vorliegt, entgegen den Befunden anderer Autoren[2] kein starker Metallchelat-Bildner ist[3]. Aus der schwachen Metall-Affinität von Rfl lässt sich die Metall-Spezifität aktiver Flavoenzyme[4,5] nicht erklären.

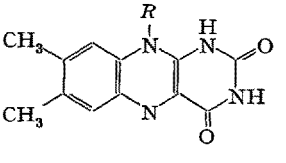
Auch die aus Rfl durch Aufnahme von 1 Mol H_2 entstehende Leukoform $RflH_2$ zeigt, wie wir fanden[6], keine potentiometrisch oder spektrophotometrisch nachweisbare Metall-Affinität.



Rfl



$RflH_2$



RflH (R = Ribityl)

In der Zwischenzeit ist man – vor allem durch die Untersuchungen von BEINERT[7] – darauf aufmerksam gemacht worden, dass ein dritter Flavin-Strukturtyp, die Flavosemichinone RflH[8], für die Biokatalyse ausschlaggebende Bedeutung hat.

Zu den Faktoren, welche diese radikalische Flavin-Struktur *in vivo* stabilisieren, gehört einmal, wie durch HARBURY *et al.*[9] experimentell begründet und von ISEN-

[1] P. HEMMERICH und S. FALLAB, Helv. chim. Acta *41*, 498 (1958).
[2] A. ALBERT, Biochem. J. *54*, 646 (1953); *47*, xvii (1950).
[3] Eine Ausnahme bilden Ag- und Hg-Ionen, vgl. [11], [19].
[4] D. J. D. NICHOLAS, Nature *179*, 800 (1957).
[5] H. R. MAHLER, Adv. Enzymol. *17*, 233 (1956).
[6] pH-Titration unter H_2 in Gegenwart von Pd auf Silicagel.
[7] H. BEINERT, J. biol. Chem. *225*, 479 (1957); Biochem. biophys. Acta *20*, 589 (1956).
[8] Vgl. L. MICHAELIS und G. SCHWARZENBACH, J. biol. Chem. *123*, 538 (1938).
[9] H. A. HARBURY und K. A. FOLEY, Proc. nat. Acad. Sci., Wash. *44*, 662 (1958). – H. A. HARBURY, K. F. LANOUE, P. A. LOACH und R. M. AMICK, Proc. nat. Acad. Sci., Wash. *45*, 1708 (1960).