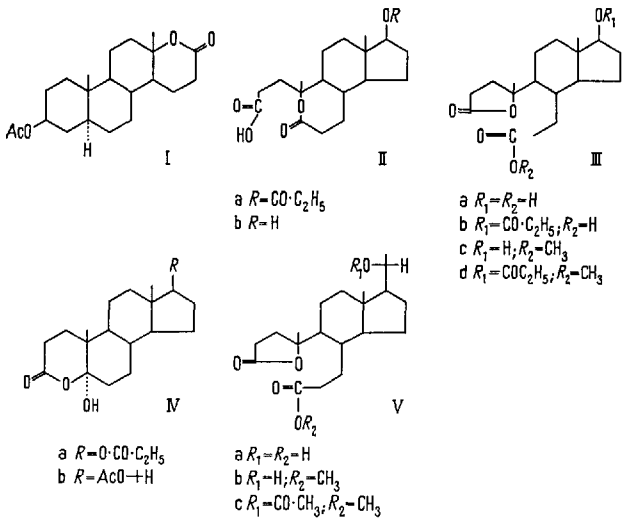
The unstable saponification product IIa had bands at 1750 and 1715 cm^{-1}. On the other hand the γ-lactone IIIa showed bands at 1770 and 1705 cm^{-1}. Again the 19-methyl exhibited the characteristic downfield shift (τ 8.64) in the NMR spectrum.



Similar results were obtained with 20β-acetoxypregn-4-en-3-one. The acidic syrupy reaction product was directly saponified to yield Va, m.p. 220–222°. Its structure was proved by alternative synthesis from IVb, m.p. 186–189°. Treatment of IVb with acetic acid-hydrogen peroxide followed by saponification, gave Va. Esterification with diazomethane gave Vb, m.p. 177–180°, which was acetylated to Vc, m.p. 164–165°.

It is reasonable to assume that the lactone II arises from the lactol IV. The mode of formation of IV is not immediately apparent. We have previously demonstrated that Baeyer-Villiger oxidation of Δ^4-3-ketones yields 7-membered enol-lactones (3-oxo-3a-oxa-4-enes) which can give 4,5-epoxides[7]. Similar enol-lactones have been shown to be formed from 4-ene-3-bis-hydroperoxides[8]. Formation of 4,5-diols from such enol-lactones (or epoxides), and loss of the elements of formic acid would lead to IV. Alternatively, 3-keto-4,5-glycol formation from 4-en-3-ones may be the initial step. A mode of action leading from 7-membered enol lactones to 3,5 lactones (3-oxo-4-oxa compounds)[9] will have to be ruled out since such lactones would be stable under the conditions employed by us.

The method described provides a means for the facile elimination of an α-carbon in a conjugated carbonyl system, and concomitant insertion of an oxygen function in the resulting ketonic product.

The utility of the procedure is further extended by the observation that an acetyl moiety, i.e. in progesterone is not being attacked, while the oxidation of ring A proceeds as described. Isolated double bonds give glycols as evidenced by the conversion of pregnenolone to the 3β,5α,6β-trihydroxypregnan-20-one.

Zusammenfassung. Oxydation von steroidalen Δ^4-3-Ketonen mit Wasserstoffsuperoxid in Anwesenheit von Selendioxid führt zu Verbindungen der allgemeinen Struktur II. Diese Methode erscheint besonders geeignet für eine oxydative Eliminierung des α-Kohlenstoffatoms in konjugierten Ketonen, sowie für die Synthese von Norsecosäurelactonen II. Ausgehend von 17-Ketonen erhält man Laktone I. Ein möglicher Reaktionsmechanismus wird diskutiert.

E. Caspi and S. N. Balasubrahmanyam

Worcester Foundation for Experimental Biology, Shrewsbury (Massachusetts, U.S.A.), March 18, 1963.

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d-Lysergyl-L-valin-methylester, ein neues, natürliches Mutterkornalkaloid[1]

Bei der Aufarbeitung von Alkaloidmutterlaugen aus Zuchtmutterkorn wurde ein neues, niedrigmolekulares Mutterkornalkaloid aufgefunden, das dann auch als Spurenalkaloid in vielen natürlichen Mutterkorndrogen nachgewiesen werden konnte. Diese neue Verbindung erwies sich als *d*-Lysergyl-L-valin-methylester. Sie stellt einen Mutterkornalkaloidtyp dar, von dem schon zahlreiche Vertreter auf partialsynthetischem Weg hergestellt worden waren[2] und der jetzt erstmals in der Natur beobachtet wurde. Das neue Alkaloid konnte aus den Alkaloidmutterlaugen durch wiederholtes Chromatographieren an neutralem Aluminiumoxid mit Benzol, dem steigende Mengen Methanol zugesetzt wurden, isoliert werden. Die freie Base ist in den meisten organischen Lösungsmitteln leicht löslich und konnte bisher nicht zur Kristallisation gebracht werden.

Amorphe Base Smp 80–85°. $[\alpha]_D^{20} = -103°$ ($\pm 2°$) (c = 1,6 in Chloroform), $[\alpha]_D^{20} = -65°$ ($\pm 2°$) (c = 1,4 in Pyridin). Für die Analyse wurde im Hochvakuum bei 100° getrocknet (Schmelze). $C_{22}H_{27}O_3N_3$ Ber. C 69,3 H 7,1 O 12,6 N 11,0%; Gef. C 69,0 H 7,2 O 12,6 N 11,2%. UV-Spektrum in Methanol: Maxima bei 225 mμ (4,34) und 311 mμ (3,94); Minimum bei 269 mμ (3,34). Titration mit 0,05*N* HCl: Mol-Gew. Ber. 381,5 Gef. 384/385. Gibt blaue Keller- und van Urk-Farbreaktion.

Das Hydrogenmaleinat kristallisiert aus Aceton in feinen Nadeln. Smp. 185° (Zers.). $[\alpha]_D^{20} = +38°$ (c = 1,2 in Alkohol/Wasser 1:1).

Das neue Alkaloid lässt sich durch Kochen der methanolischen, schwach essigsauren Lösung in *d*-Isolysergyl-L-valin-methylester umlagern. Aus dem Isomerengemisch lässt sich die Isoverbindung durch Chromatographieren an neutralem Aluminiumoxid (Aktivität I) mit Benzol + 0,2% Methanol als schneller wandernde Zone abtrennen.

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Aus Aceton kristallösungsmittelfreie Prismen. Smp. 167° (Zers.) $[\alpha]_D^{20} = +421°$ ($\pm 4°$) (c = 2,0 in Chloroform) $[\alpha]_D^{20} = +483°$ ($\pm 4°$) (c = 2,5 in Pyridin). Für die Analyse wurde die Substanz bei 100° im Hochvakuum getrocknet. $C_{22}H_{27}O_3N_3$ Ber. C69,3 H7,1 O12,6 N11,0%; Gef. C69,3 H7,0 O12,7 N11,2%.

Unter energischen Bedingungen, Kochen in 10prozentiger KOH, wird das Alkaloid zu Lysergsäure und Valin verseift. Unter mildern Bedingungen, durch Stehenlassen in 0,1 *N* KOH bei 20° lässt sich das neue Alkaloid zu *d*-Lysergel-L-valin hydrolysieren. Nach zweimaligem Umkristallisieren, durch Lösen in verdünntem Ammoniak und Ansäuern mit Essigsäure auf pH 5,5, war das Produkt papierchromatographisch rein und in allen seinen Eigenschaften mit einem synthetisch dargestellten Vergleichspräparat identisch. Smp. 250° (Zers.). $[\alpha]_D^{20} = +60°$ ($\pm 3°$) (c = 0,5 in 2 *N* Ammoniak). Für die Analyse wurde bei 120° im Hochvakuum getrocknet. $C_{21}H_{25}O_3N_3$ Ber. C68,6 H6,9 N11,4%; Gef. C68,6 H7,0 N11,7%. Titration mit 0,02 *N* NaOH: Mol-Gew. Ber. 367,4 Gef. 372. Durch Verestern von *d*-Lysergyl-L-valin mit Diazomethan gelangte man wieder zu der mit dem natürlichen Alkaloid identischen Verbindung.

Summary. A description is given of the isolation from ergot of *d*-Lysergyl-L-valine methyl ester, a new minor alkaloid.

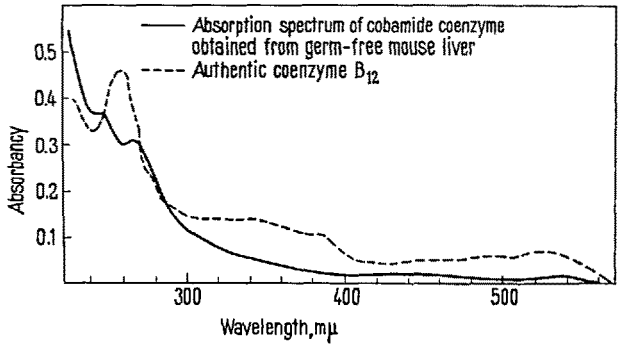
W. SCHLIENTZ, R. BRUNNER und A. HOFMANN

Pharmazeutisch-chemische Forschungslaboratorien, Sandoz AG, Basel (Schweiz), 24. Juni 1963.

Isolation of Coenzyme B_{12} from Liver of Germ-free Mice

Recent reports from BARKER's laboratory[1,2] have summarized studies leading to the discovery and characterization of several forms of cobamide coenzymes which are light-sensitive derivatives of vitamin B_{12} containing an adenosyl moiety attached to the cobalt atom in the corrin ring. The obligatory participation of cobamide coenzymes has been demonstrated in the enzyme-catalyzed isomerization of glutamate to β-methylaspartate[3], the conversion of methylmalonyl-coenzyme A to succinyl-coenzyme A[4–6], and the dismutation of 1,2-diols to the corresponding deoxyaldehyde[7]. The synthesis of B_{12} coenzymes by enzyme preparations obtained from several bacterial species has been amply demonstrated[8–11], and the presence of cobamide coenzymes in human, chicken, lamb and rabbit liver has been reported[12]. Recently the conversion of Co^{60}-labeled cyanocobalamin to its coenzyme form has been observed in the rabbit *in vivo*[13]; however, there has been no direct demonstration of the enzymatic synthesis of B_{12} coenzymes in preparations obtained from mammalian tissue *per se*. While the existence of such synthesis seemed likely, the possibility still remained that the formation of these materials might occur exclusively through the activity of intestinal flora, in view of the facile synthesis of cobamide coenzymes catalyzed by extracts of certain microorganisms. It seemed reasonable, therefore, to examine the livers of animals raised under germ-free conditions for the presence of B_{12} coenzymes.

The germ-free animals studied were drawn from a colony of white Swiss mice which has been maintained in steel isolators in the LGAR for approximately $4^1/_2$ years. The colony was obtained originally from the Lobund Institute, University of Notre Dame. The animals are fed a semi-synthetic diet[14] which is sterilized by steam at 255°F for 25 min. They are checked approximately every 2–3 weeks for bacteria, fungi, and parasites according to techniques described previously[15]. The control mice used for comparison were drawn from a colony which is maintained in an ordinary animal room, and which harbors a varied flora. This colony was derived from the germ-free colony. Breeders from the latter are removed periodically from the isolators and added to the control colony in an effort to keep the gene pools similar. These animals are fed the same sterilized diet given the germ-free.



Absorption spectrum of cobamide coenzyme isolated from germ-free mouse liver and authentic coenzyme B_{12}. The solution was adjusted to a final concentration of 0.01 *M* potassium phosphate buffer at pH 6.7. An exact comparison of the spectrum of the cobamide coenzyme obtained from the germ-free mouse liver tissue and crystalline coenzyme B_{12} cannot be made at this stage of purification[12]. The spectum shown here compares favorably with those reported by TOOHEY and BARKER[12] for the coenzymes obtained from lamb, human and rabbit liver.

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