Older female phase individuals with ripe oocytes show again a few spermatocytes and sperms among oocytes which are ready to be laid. Sperms are always very scarce and they form small groups which are often adjacent to the oocytes (Fig. 2). Spermatocytes are generally found in clusters along the intestinal wall. The rapid secondary spermatogenesis is thus responsible for the subsequent self-fertilisation in *O. labronica*.

Similar processes were demonstrated by COGNETTI in the Asteroid species *Asterina pancerii*[8].

Researches are now being carried on in order to ascertain whether primary and secondary spermatogenesis takes place in all individuals of *O. labronica*.

U. PARENTI

Istituto di Zoologia, Università di Modena, and Acquario di Livorno (Italy), June 16, 1960.

Riassunto

Ophryotrocha labronica (BACCI e LA GRECA)[1] è ermafrodita proterandrica e si dimostra che molti individui presentano una fugace spermatogenesi secondaria che rende possibile l'autofecondazione.

Isolierung von Lysergsäure-Alkaloiden aus der mexikanischen Zauberdroge Ololiuqui (*Rivea corymbosa* (L.) Hall. f.)

Die Samen von *Rivea corymbosa* (L.) Hall. f., der alten aztekischen Zauberdroge «Ololiuqui»[1] und von *Ipomoea tricolor Cav.* (*Convolvulaceae*) werden auch heute noch von manchen Indianerstämmen Südmexikos für magische Zwecke verwendet.

R. G. WASSON verdanken wir Muster dieser beiden Drogen, die in Oaxaca (Mexiko) gesammelt worden waren. Durch schonende Extraktion konnte aus beiden Samen Alkaloidfraktionen gewonnen werden, die im Dünnschicht-Chromatogramm mindestens 5 übereinstimmende Indolverbindungen erkennen liessen. Es gelang, die 3 Hauptkomponenten zu kristallisieren und chemisch zu identifizieren.

Substanz A: Aus Methanol kristallösungsmittelfreie Prismen, Smp. 232–240° (korr.) Zers., $[\alpha]_D^{20} = +105° (\pm 7°)$ ($c = 0{,}1$ in Chloroform/Methanol 1 : 1). Gefunden: C 71,8; H 6,6; O 6,2; N 15,5%. Berechnet für $C_{16}H_{17}ON_3$ (267,3): C 71,9; H 6,4; O 6,0; N 15,7%. UV-Spektrum: Maxima bei 240 mμ ($\log \varepsilon_{max} = 4{,}3$) und bei 311 mμ ($\log \varepsilon_{max} = 3{,}9$). Dieses Spektrum ist mit demjenigen der Lysergsäure und Isolysergsäure praktisch identisch[2]. Diese physikalisch-chemischen und analytischen Daten entsprechen den Eigenschaften von d-Lysergsäure-amid. Die vollkommene Übereinstimmung der IR-Spektren von Substanz A und von d-Lysergsäure-amid (Iso-ergin)[3] bestätigt die Identität dieser beiden Verbindungen.

Substanz B: Aus Methanol kristallösungsmittelhaltige Prismen vom Smp. 136°(korr.), $[\alpha]_D^{20} = +383° (\pm 6°)$ ($c = 0{,}1$ in Chloroform/Methanol 1 : 1). Die im HV bei 120° getrocknete Verbindung lieferte folgende Analysenwerte: C 71,6; H 6,6; O 6,2; N 15,4%. Berechnet für $C_{16}H_{17}ON_3$ (267,3): C 71,9; H 6,4; O 6,0; N 15,7%. UV-Spektrum: identisch mit demjenigen von Substanz A. Diese Daten stimmten auf d-Isolysergsäure-amid (Ergin)[4]. Die Identität von Substanz B mit Ergin wurde durch die übereinstimmenden IR-Spektren bestätigt.

Substanz C: Aus Aceton massive kristallösungsfreie Polyeder vom Smp. 213–215° (korr.) Zers., $[\alpha]_D^{20} = -195°$ ($c = 0{,}2$ in Pyridin). Gefunden: C 74,6; H 7,9; O 6,5; N 11,0%. Berechnet für $C_{16}H_{20}ON_2$ (256,3): C 74,9; H 7,9; O 6,2; N 10,9%. UV-Spektrum: Maxima bei 222 mμ ($\log \varepsilon_{max} = 4{,}6$), bei 282 mμ ($\log \varepsilon_{max} = 3{,}9$) und bei 291 mμ ($\log \varepsilon_{max} = 3{,}8$). Diese Daten stimmten mit den Eigenschaften von Chanoclavin[5] überein. Die Identität von Substanz C mit diesem erstmals aus saprophytischen Kulturen des Mutterkornpilzes von *Pennisetum typhoideum* Rich. isolierten Alkaloid wurde durch den Vergleich der IR-Spektren, die vollkommene Übereinstimmung zeigten, bestätigt.

Das Vorkommen von Lysergsäure-Alkaloiden, die bisher nur in niederen Pilzen der Gattung *Claviceps* gefunden wurden, in der Pflanzenfamilie der *Convolvulaceae* ist ein unerwarteter, phytochemisch interessanter Befund.

Die Analysen wurden im mikroanalytischen Laboratorium SANDOZ (Leitung Dr. W. SCHÖNIGER) ausgeführt, die Spektren in der spektral-analytischen Abteilung (Leitung Dr. H. G. LEEMANN) aufgenommen. Für wertvolle Diskussion der Spektren danken wir Herrn Dr. H. G. LEEMANN bestens.

A. HOFMANN und H. TSCHERTER

Pharmazeutisch-chemisches Laboratorium SANDOZ, Basel, 28. Juli 1960.

Summary

The psychotomimetic active principles of the ancient aztec drug «Ololiuqui» (*Rivea corymbosa* (L.) Hall. f.) have been found to be alkaloids of the ergot type. Three of the components could be crystallized and identified, i.e. ergine (isolysergic acid amide), isoergine (lysergic acid amide) and chanoclavin. The same alkaloids were found in the seeds of the related convolvulaceous vine *Ipomoea tricolor* Cav.

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Further Studies on Corticotropin Releasing Factor (CRF); Corticotropin Releasing Activity of Synthetic Peptides

In a preceeding communication[1] we have reported that it was possible to obtain, from hog posterior pituitary powder, a fraction free of vasopressin and yet having a strong CRF activity. By using the method of assay that we have described[1,2], we have now obtained peptide fractions of which 0.5–1.0 μg is as active as 4 μg of a standard preparation called CRF 91. These fractions have been obtained through the following operations: (1) Extraction of the posterior pituitary powder with dilute acetic acid; (2) Fractionated precipitation with acetone; (3) Countercurrent distribution according to the classical method of Craig; (4) Zone electrophoresis according to the method

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Substances	CRF-activity Versus 4 μg of CRF 91 [a]	MSH activity U/g
Natural		
Fraction 3	1.0 μg : 1.3	$5—6\times 10^{8}$ [6]
Fraction 4	1.0 μg : 1.3	
Fraction 5	1.0 μg : 0.8	
Synthetic		
N-ε-formyl-α-MSH [b]	4 μg : 0.7	2×10^{10} [9]
Hexapeptide [c]	4 μg : 1.2	2×10^{5} [6]
Heptapeptide	0.1 μg : 1.0	2.8×10^{5} [6]
Decapeptide	0.1 μg : 1.0	—

[a] As explained in ref. [1] and [2], one determines the amount of unknown substance, giving a release of ACTH equal to that given by 4 μg of the standard CRF 91: if we take for instance fraction 3, we see that 1 μg of this fraction provokes a release of ACTH equal to that produced by 1.3×4 μg of CRF 91, and that 0.1 μg of the heptapeptide produces a release equal to that given by 1.0×4 μg of CRF 91, etc. In the latter example, we would say that the heptapeptide is about 40 times more active than CRF 91; but this assumption can be made only when the ratio $x/4$ μg CRF 91 is equal to 1.0.

[b] N-ε-formyl-α-MSH: Ac.Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.Try.Gly.Lys.Pro.Val(NH_2)
|
N-ε-formyl.

[c] Hexapeptide: NH_2
|
H.Glu.His.Phe.Arg.Try.Gly.OH

Heptapeptide: NH_2
|
H.Met.Glu.His.Phe.Arg.Try.Gly.OH

Decapeptide: NH_2
|
H.Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.Try.Gly.OH

of PORATH *et al.*[3]. The 3 first steps have already been described[1]; we describe here only the 4th step:

The material coming from the counter current distribution (25 mg) was dissolved in 1 ml of 0.1 *N* acid and put on a cellulose column of 0.6×25 cm; dinitrophenyl-ethanolamine was used as a tracer and the run was performed in 0.6 *M* pyridinium acetate; 0.2% phenol was added as a preservative and the temperature of the buffer was maintained at about 10–12°C with circulating water. The electrophoresis was run under 30 mA and 300 volts. Fractions of 2 ml were collected every 20 min and ninhydrin analysis was performed on 0.2 ml samples after acid hydrolysis (HCl 15 h in sealed tubes at 100°C).

In this manner several fractions were obtained numbered 1, 2, 3, 4, etc., the first one appearing after 12 h. The fraction 2 consists mainly of the tripeptide Leu. Arg. Leu; the CRF activity was found in peaks 3 and 4 (see Table). These 2 active fractions (3 and 4) represent approximately 10% of the material put on the column. Fractions 3 and 4 were also analyzed for their amino-acid composition: fraction 3 has a composition similar to that of the α-Melanophore Stimulating Hormone (α-MSH) given by HARRIS[4], with the exception that threonine and leucine were present.

The identity between α-MSH and CRF has been envisaged for a while by ourselves and by GUILLEMIN *et al.*[5]. This identity does not seem possible now for the following reasons:

- the presence of threonine and leucine in the molecule of the peptide(s) possessing CRF activity,
- the fact that the active fraction 3 has an MSH activity amounting only to 1–2.5% of that of pure α-MSH; this weak MSH activity may be either intrinsic to the material or may represent some contamination with α-MSH[6],
- the fact that highly purified natural α-MSH[7], as well as synthetic formyl-α-MSH, kindly supplied by Dr. K. HOFMANN, have no significant CRF activity.

In the course of these experiments we have studied several synthetic peptides related to the group of the Corticotropins and the Melanophore Stimulating Hormones, generously supplied by Drs. SCHWYZER and KAPPELER[8]. *We have found that several of these peptides, having a sequence in common with the Corticotropins and the MSH have a strong in vitro CRF activity, higher than that of the most active natural fractions so far obtained by us* (*Table*).

M. PRIVAT DE GARILHE, C. GROS, J. PORATH, and EVA-BRITA LINDNER

Laboratoire de Chimie biologique de la Faculté des Sciences, Paris (*France*) *and Biokemiska Institutionen, Uppsala* (*Sweden*), *June 1, 1960.*

Résumé

Une fraction peptidique ayant une forte activité d'hypophyso-stimuline à effet corticotrope ou «Corticotropin Releasing Factor» (CRF), *in vitro*, a été obtenue à partir de la poudre d'hypophyses postérieures de porc par les opérations suivantes:

1. Extraction de la poudre avec l'acide acétique dilué; 2. Précipitation fractionnée à l'acétone; 3. Distribution à contre courant; 4. Electrophorèse de zones.

La constitution en amino acides de cette fraction est très voisine de celle de l'α-MSH; cependant α-MSH et CRF ne sont pas identiques pour des raisons chimiques et physiologiques. Bien que l'α-MSH n'ait pas d'activité CRF significative, des peptides synthétiques ayant une séquence commune avec l'α-MSH possèdent cette activité.

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[9] K. HOFMANN, personal communication.

Synthetic Peptides Related to the Corticotropins (ACTH) and the Melanophore Stimulating Hormones (MSH) Possessing Corticotropin Releasing Activity (CRF-Activity)

Our synthetic work in the field of β-MSH[1] required the synthesis of a number of peptides with amino-acid sequences common to ACTH, α-MSH, and β-MSH. Among these were the pentapeptide (I), the hexapeptide (II), and the heptapeptide (III). The latter compound contains the whole amino-acid sequence common to all three hormones of the pituitary mentioned above.

LI[2] and HARRIS[3] have put forward the hypothesis that the common sequence might have some special biological

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