Quantitatively they synthesized 0.08 µg pantothenic acid and 0.18 µg niacin/ml of culture medium in 48 h. They also synthesized 0.9 mg ascorbic acid/g of mannose in 3 h. This amount was reduced to 0.1 mg/g mannose, when mycetomal tissue homogenates were treated with 1% kanamycin. PIERRE[5] reported such a synthesis by the symbiotes of *Leucophaea maderae* (F). However the utility of such a synthesis to insects could not be demonstrated, as neither a holidic diet for the bug could be formulated nor could the insect be made aposymbiotic.

Attempts to produce aposymbiotic bugs by treating the insects with various concentrations of streptomycin, chloramphenicol, tetracyclines, penicillins, kanamycin and sulphadiazine, either injected with glass capillary needles or by feeding the bugs on soaked seeds and on dipped twigs of the host plant, were unsuccessful. Mixture with dimethyl sulphoxide was of no value. Centrifugation of eggs (18,000 rpm for 20 min, 10,000 rpm for 30 min) did not yield any result. Lysozyme could not inhibit the multiplication of bacteria in culture media. Haemolymph taken out aseptically from kanamycin-treated bugs (600 µg/g body weight), 4 h after injection, did not produce any inhibition zones to plated cultured symbiotes, showing degradation of the antibiotic. The bacterial symbiotes were not killed by dipping the mycetomal tissue for 1 h in tubes of 1% kanamycin solution or 2% ledermycin, and turbidity appeared on inoculation in nutrient broth.

Cultured symbiotes were found to degrade 29 µg of DDT. 820 µg of parathion and 18 µg of carbaryl, when definite quantities of these insecticides in acetone solution were incubated with symbiotes in nutrient broth for 6 h. Such degradation by the cultured symbiotes of apple maggot, was also studied by MALLORY and MATASMURA[6]. Full details of these findings will be published elsewhere[7].

Zusammenfassung. Es wurden symbiontische Bakterien aus der Wanze *Cletus signatus* Walker isoliert, in vitro kultiviert und als neue Var. *signatus* von *Bacillus cereus* bestimmt.

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Ribosomal Proteins from the Liver of the South American Rattlesnake, *Crotalus durissus terrificus*

The structure of eukaryotic ribosomes has been widely investigated in the last few years[1]. However, little is known on this subject in Reptilia[2]. This paper reports studies on the ribosomes of the reptile *Crotalus durissus terrificus*, with special emphasis on the ribosomal proteins.

Material and methods. South American rattlesnakes of both sexes (200–300 g body weight) were used in all experiments. Ribosomes and ribosomal subunits were obtained from the liver as described in a previous paper[2]. Only preparations with $A_{260\,nm}/A_{235\,nm}$ and $A_{260\,nm}/A_{280\,nm}$ ratios of about 1.50 and 1.85, respectively, were used[3]. Protein and RNA contents of the ribosomes were measured as reported by FRIEDMAN et al.[4]. The sedimentation coefficient of the monosomes was determined by sucrose-density-gradient centrifugation under the conditions described previously[2]. Rat liver ribosomes, isolated according to MOLDAVE and SKOGERSON[5], were used as a marker. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of the ribosomal proteins was carried out by the procedure of BICKLE and TRAUT[6]. The buffer for electrophoresis contained 0.1*M* sodium phosphate, pH 7.2, and 0.1% sodium dodecyl sulphate. Gels were stained[6] with Comassie brilliant blue and scanned at 600 nm in a Beckman model Acta III spectrophotometer. The molecular weights of the ribosomal proteins were estimated as a function of their relative mobilities[7], using bovine serum albumin, ovalbumin, chymotrypsinogen and cytochrome c as standards.

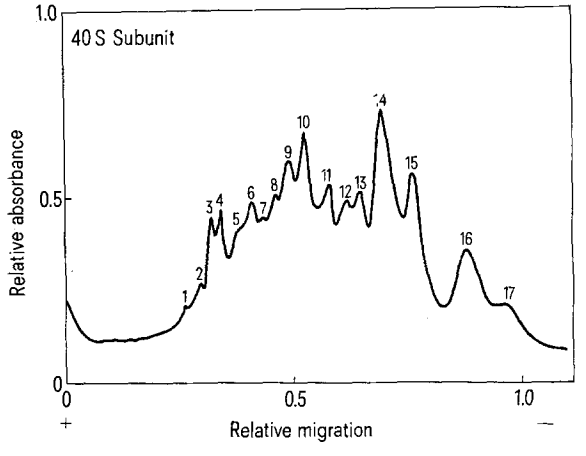

Fig. 1. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of ribosomal proteins from the 40 S subunit. Electrophoretic analysis was carried out on a 10% gel at 8 mA per tube at room temperature until the bromophenol blue band had just reached the end of the gel.

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Results and discussion. The results from sucrose gradient centrifugation, using rat liver ribosomes as a marker, showed that most of the ribosomes from rattlesnake liver sediment as 80S monosomes. It was also found that the ribosomes of this reptile comprise 49% RNA and 51% protein, giving a RNA/protein ratio of about 0.96.

The electrophoretic profiles of the ribosomal proteins from the 40 S and 60 S subunits are shown in Figures 1 and 2, respectively. The purity of the ribosomal subunits was always checked by recentrifugation on sucrose gradients prior to electrophoresis of the proteins on polyacrylamide gels. The electrophoretic analysis was performed with subunits treated directly with sodium dodecyl sulphate. Discrepancies concerning the characterization of ribosomal proteins have been attributed[8] to the different methods used to extract and solubilize these proteins. It should be emphasized, however, that the extraction with acetic acid[9] and with LiCl[10] gave results similar to those shown in the Figures 1 and 2. The possibility that bands observed in these electrophoretic profiles were not of ribosomal origin was reduced by the washing of the ribosomes with a high-salt buffer[11].

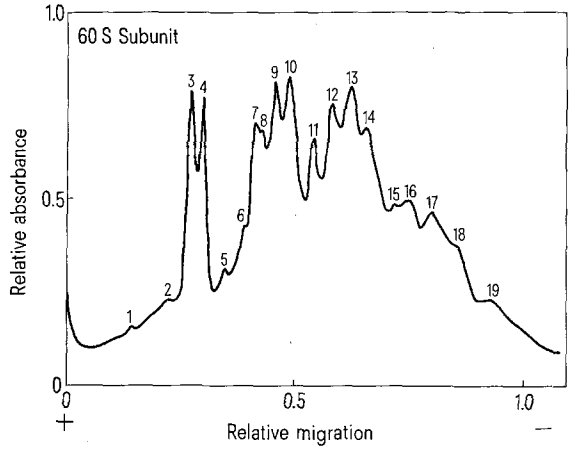

Fig. 2. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of ribosomal proteins from the 60 S subunit. Electrophoretic analysis was performed under the same conditions described in Figure 1.

Molecular weights of ribosomal proteins from 40 S and 60 S subunits

Band number	Molecular weight ($\times 10^{-3}$)	
	40 S	60 S
1	58.0	78.5
2	52.0	60.5
3	48.0	56.0
4	45.5	52.0
5	41.0	44.0
6	38.0	41.0
7	34.5	38.0
8	32.5	36.0
9	29.5	33.0
10	27.0	29.5
11	23.0	26.0
12	20.5	23.5
13	19.0	20.5
14	16.5	19.0
15	14.0	16.5
16	10.5	14.5
17	9.0	13.0
18		11.0
19		10.0

Values represent means of 2 independent determinations.

The molecular weights of the ribosomal proteins were mostly in the range from 10 to 45×10^3 daltons for both subunits (Table). These values are in agreement with those observed for other eukaryotic ribosomal proteins[12–14]. In previous reports[2,15,16] from this laboratory, it was found that the RNA components of rattlesnake ribosomes have the same sedimentation coefficients as those observed for other vertebrate animals, 28 S, 18 S and 5 S, corresponding to molecular weights of 1.70×10^6, 0.8×10^6 and 0.4×10^5 daltons, respectively[17]. Adding these values to the sums of the molecular weights of the ribosomal proteins listed in the Table, one can estimate for the 40 S subunit a particle weight of 1.32×10^6 daltons, in agreement with that reported for the small subunit of rat liver ribosomes[18]. The corresponding estimate for the 60 S subunit (2.36×10^6 daltons) is lower than reported for other eukaryotes[19], but this may be explained by the fact that some bands observed in Figure 2 comprise more than one distinct ribosomal protein. Since this may also be true in the case of the 40 S subunit (Figure 1), further studies are required using two-dimensional electrophoresis[20]. Another possible explanation is that at least some proteins have variable copies per ribosomal subunit. It is noteworthy that reports[1,14,21] concerning the stoichiometry of ribosomal proteins in animal cells are conflicting, and this is even true in the well established *Escherichia coli* system[8,22].

The weight-average molecular weight of the ribosomal proteins was calculated by dividing the area under the electrophoretic profiles (Figures 1 and 2) into equal halves[21]. Values of 22,000 and 26,000 daltons were obtained for the proteins from the 40 S and 60 S subunits, respectively. These data are close to that observed for unfractionated ribosomal proteins from rat liver[18].

Finally, the results presented here support the idea[6,12] that the increased protein content of eukaryotic ribosomes over bacterial ribosomes is due to the presence of proteins with larger molecular weights.

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Zusammenfassung. In Ribosomen von Klapperschlangenleber ist das Verhältnis RNS/Protein 0,96, und das Molekulargewicht der meisten ribosomalen Proteine liegt zwischen 10 und 45×10^3 Daltons. Das durchschnittliche Molekulargewicht ist 22000 bzw. 26000 Daltons für die Proteine der 40 S bzw. der 60 S Untereinheit. Diese Daten weisen darauf hin, dass der erhöhte Proteingehalt der Ribosomen dieses Reptils gegenüber Ribosomen von Bakterien durch das Vorhandensein von Proteinen mit höherem Molekulargewicht bedingt ist.

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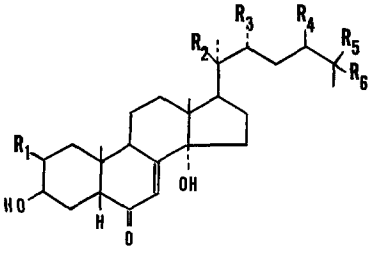
Biological Activity of Insect Ecdysones and Analogues in vitro

The relationship between the structure of ecdysone analogues and their biological activity was investigated with in vivo bioassays in both Diptera [1] and Lepidoptera [2, 3]. However, some ecdysone analogues were converted to insect ecdysones in vivo [4]. Hence, the biological activity of some of the analogues may depend upon their conversion to active molecules in the insect [5]. The relationship between structure and activity should be examined in an in vitro system in which metabolism of the ecdysones is less likely to occur [6–8]. We have reported previously that continuous exposure to α-ecdysone and β-ecdysone had different effects on morphogenesis and cuticle deposition in imaginal disks in vitro [9–11]. Furthermore, fat body modified the action of the ecdysones [10–12]. We report here on relative activity of insect ecdysones and various analogues in vitro (Figure) in bioassays of wing disks of *Plodia interpunctella* (Hübner).

Wing disks from last-instar larvae were cultured as previously described [11]. The hormone analogues were tested as a continuous exposure in cultures of wing disks with and without fat body. Also, each analogue was tested as a 24-h pulse on cultures that did not contain fat



Compound	R_1	R_2	R_3	R_4	R_5	R_6
α-Ecdysone	OH	H	OH	H	CH_3	OH
β-Ecdysone	OH	OH	OH	H	CH_3	OH
Ponasterone A	OH	OH	OH	H	CH_3	OH
Inokosterone	OH	OH	OH	H	CH_2OH	H
Podecdysone A	OH	OH	OH	C_2H_5	CH_3	OH
2-Deoxycrustecdysone	H	OH	OH	H	CH_3	OH
22,25-di-Deoxyecdysone	OH	H	H	H	CH_3	H

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Table I. Effects of continuous exposure to ecdysone analogues on wing disks in vitro

Compound	Lowest concentration (μg/ml) required to produce response[a]			
	No fat body		Fat body	
	Cuticle	Evagination	Cuticle	Evagination
α-Ecdysone	—	0.1 (50%)	10.0 (90%)	0.1 (100%)
β-Ecdysone	1.0 (17%)	0.1 (90%)	0.1 (100%)	0.1 (100%)
Ponasterone A	—	0.1 (50%)	0.1 (25%)	0.1 (100%)
Inokosterone	—	0.1 (55%)	0.1 (20%)	0.1 (100%)
2-Deoxycrustecdysone	10.0 (15%)	0.1 (100%)	1.0 (45%)	0.1 (100%)
22,25-di-Deoxyecdysone	—	1.0 (100%)	—	1.0 (100%)
Podecdysone A	—	—	—	1.0 (100%)

[a] Percent response is given in parentheses. The concentrations tested were 0.1, 1.0, and 10.0 μg/ml.