Table 1. Effect of tubulosine on protein and polyphenylalanine synthesis in cell-free preparations from different species. Reticulocytes: Hemoglobin synthesis was measured in reaction mixtures containing, in a final volume of 0.5 ml, 5 μmole of glutathione; 33 μmole of KCl; 16 μmole of tris-HCl, *p*H 7.4; 3.3 μmole of $MgCl_2$; 5 μmole of phosphoenolpyruvate; 0.033 μmole of guanosine triphosphate (GTP); 1.5 μg of pyruvate kinase; 93 μg (11,980 count/min) of ^{14}C-leucyl-sRNA; 0.33 μmole of ^{12}C-L-leucine; amino acid mixture containing 0.05 μmole of each of the following L-amino acids: alanine, arginine, aspartic acid, glycine, glutamine, glutamic acid, histidine, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, hydroxyproline, cysteine, and valine; 0.3 mg of reticulocyte ribosomes, and tubulosine at the final concentration shown. Reactions mixtures were incubated at 37°C for 5 minutes. *Saccharomyces fragilis*: Polyphenylalanine synthesis was measured in reaction mixtures containing, in a final volume of 0.5 ml, 50 μmole of imidazole, *p*H 7.0; 3 μmole of magnesium acetate; 50 μmole of NH_4Cl; 0.4 μmole of spermine; 1 μmole of GTP; 50 μg of polyuridylic acid; 70 μg of ^{14}C-phenylalanyl-sRNA (4950 count/min); 2 μmole of ^{12}C-L-phenylalanine and 0.34 mg of *Saccharomyces fragilis* ribosomes. Reaction mixtures were incubated at 25°C for 10 minutes. *Escherichia coli*: The reaction mixture for polyphenylalanine synthesis was composed of 25 μmole of tris-HCl, *p*H 7.4; 5.5 μmole of magnesium acetate; 15 μmole of KCl; 1.5 μmole of adenosine triphosphate; 0.1 μmole of GTP; 5.6 μmole of mercaptoethanol; 5 μmole of phosphoenolpyruvate; 0.02 μmole of each of the 19 amino acids listed above; 0.02 μmole of ^{14}C-phenylalanine (191,000 count/min); 16 μg of pyruvate kinase; 8 μg of polyuridylic acid, and 0.5 mg of preincubated S-30 fraction (*3*) in a final volume of 0.5 ml. Reaction mixtures were incubated at 25°C for 10 minutes. All reactions were terminated by the addition of trichloroacetic acid, and radioactivity in the fraction insoluble in hot acid was then determined (*6*).

Tubulosine (mole/liter)	Rabbit reticulocytes		*S. fragilis*		*E. coli*	
	Incorporation (count/min)	Inhibition (%)	Incorporation (count/min)	Inhibition (%)	Incorporation (count/min)	Inhibition (%)
10^{-3}			125	94	15,130	0
10^{-4}	89	98	626	70	15,012	0
10^{-5}	1201	73	1522	27	15,186	0
10^{-6}	4005	10	2002	4		
10^{-7}	4461	0	2085	0		

several strains of *Entamoeba histolytica*. This ambicidal activity was compared (*12*) to that of emetine as tested by published methods (*13*); the lowest concentration of either tubulosine or emetine which produced 100 percent killing of the organisms was $1 \times 10^{-5}M$.

The data indicates that the action of tubulosine is: (i) species-specific, being active against certain mammalian cells, protozoa, and yeast but inactive against preparations of *Escherichia coli*; (ii) structurally specific, requiring a secondary nitrogen atom at the 2′-position and the (R) configuration at the 1′-carbon for activity; (iii) selective, as RNA synthesis is unaffected at concentrations of inhibitor which totally inhibit protein synthesis; and (iv) exerted during the enzymatic transfer of amino acids from aminoacyl-sRNA to the growing peptide chain. The foregoing properties are common to the ipecac alkaloids (*3*) and the glutarimide antibiotics (*4*), and they were predicted (*3*) for compounds, such as tubulosine, which contain configurational features of structure I.

The steric and electronic effects of substituents at other positions in tubulosine have not been evaluated. Nevertheless, the data reported are consistent with the structural basis assigned for the inhibition of the transfer reaction of protein synthesis (*3*) and represent a successful prediction of antibiotic activity based on structural considerations. Tubulosine should be expected to have the antitumor (*14*) and antiviral (*15*) properties shown by the ipecac alkaloids, which probably result from their action as potent inhibitors of protein biosynthesis in mammalian cells.

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16. I thank Dr. A. R. Battersby for a sample of 2′,3′-dehydro-6′-deoxytubulosine, Dr. C. J. Djerassi for deoxytubulosine, Dr. H. T. Openshaw for synthetic isotubulosine, and Dr. A. Popelak for the tubulosine. I thank Mrs. Maria Walsh for technical assistance. Supported in part by grants from the USPHS, NSF, and American Cancer Society. This is paper III in the series *Inhibitors of Protein Biosynthesis*.
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Sex Attractant of the Black Carpet Beetle

Abstract. *The principal component of the sex attractant of the black carpet beetle* Attagenus megatoma (*Fabricius*) *is* trans-*3*, cis-*5-tetradecadienoic acid.*

We report the isolation, identification, and synthesis of the principal component of the sex attractant of the black carpet beetle *Attagenus megatoma* (Fabricius) [*A. piceus* (Olivier)]. The response of the male beetle to the female has been described by Burkholder (*1*), whose laboratory bioassay was used to monitor the following isolation steps.

Thirty thousand unmated female beetles were extracted in a Waring Blendor with benzene (four 300-ml portions), and the benzene-soluble material (9.4 g) was distilled in a short-path still onto a condenser (cooled with dry ice) at 100°C and 0.01 mm-Hg. A solution of the distillate (3.8 g) in 100 ml of diethyl ether was extracted with ice-cold 0.1*N* solution of sodium hydroxide (two 100-ml portions) which, after acidification to *p*H 1.5 with 1*N* hydrochloric acid, was extracted with 200 ml of diethyl ether. The acidic fraction thus obtained (1.7 g) was chromatographed on silica gel (Gallard Schlesinger; 90 to 200 mesh; 175 g in a 3.5 by 30 cm water-cooled column); used successively were 400 ml of benzene, 800 ml of diethyl ether, 325 ml of acetone, and 600 ml of methanol. The active ether fraction (0.8 g from 17,000 female beetles) was chromatographed on an anion-exchange column [AGl-X4, chloride form, 200 to 400 mesh (Bio-Rad Laboratories); 4.6 by 28 cm column]; we used successively 500 ml of a 3 : 1 mixture of methanol and water, 550 ml of 0.1*M* solution of sodium chloride in the same mixture, and 300 ml of a saturated solution of sodium chloride in the same mixture. The active component (0.6 g), which

eluted in the first fraction, did not survive gas chromatography; it was therefore treated in diethyl ether with diazomethane, and the product was evaporatively distilled at 100°C and 0.1 mm-Hg. Activity in subsequent steps was monitored on samples that were hydrolyzed to the carboxylic acid. The distillate (0.3 g of a mixture of methyl esters from 8000 females) was fractionated by gas chromatography (5 percent SE-30 on Gas Pack F; 1 m by 4-mm-inside-diameter glass tubing; 150° to 200°C at 2°/min; He at 100 cm^3/min; 0.03 ml of a 50-percent solution in pentane per on-column injection); the active fraction was collected between minutes 5 and 13, during which time the flow of He was reduced to 50 cm^3/min. The active fraction was rechromatographed on the same column at 150°C, with He at 53 cm^3/min (0.01 ml of a 25-percent solution of pentane per injection), and the active material that eluted between minutes 10 and 22 was fractionated on a Carbowax 20-M column (4 percent on Chromosorb G; 2 m by 4-mm-inside-diameter glass tubing; 160°C; He at 51 cm^3/min; 0.01 ml of a 20-percent solution in pentane per injection). The active fraction (4 mg of the methyl ester from 8000 beetles) that eluted between minutes 24 and 30 gave a single symmetric peak on four analytical 2-m by 2-mm-inside-diameter glass columns [SE-30; Carbowax 20-M; diethylene glycol succinate; and tris (cyanoethoxy) propane].

Saponification of the methyl ester for bioassay was accomplished with a 1*N* solution of potassium hydroxide in 90-percent methanol for 2 hours at room temperature. Under these conditions, methyl myristate was quantitatively saponified.

The mass spectrum of the methyl ester of the active compound showed the following diagnostic peaks, *m/e*: 238 (*P*), 207 (*P* minus $-OCH_3$), 206 (*P* minus CH_3OH), 179 (*P* minus $COOCH_3$), 74 (CH_2COOCH_3 + H). On a double-focusing mass spectrometer, the molecular weight was 238.1908 (calculated for $C_{15}H_{26}O_2$:238.19327). The infrared spectrum (neat) showed the following diagnostic peaks (μ): 3.33 (C=CH), 5.74 (C=O), 6.03, 6.18 (weak, C=C conjugated), 8.00, 8.35, 8.60 (C—O, methyl ester), 9.85, 10.18, 10.54 (characteristic pattern for *cis-trans* conjugated double bonds, the 9.85 band being weak), 13.90 (weak CH_2 rock). The ultraviolet spectrum (pentane) was: λ_{max}, 232 mμ; ϵ, ~ 29,000. The nuclear magnetic resonance spectrum (CCl_4; τ) was: 3.55 to 4.90 (4 conjugated olefinic protons), 6.41 (3 protons; singlet; $COOCH_3$), 6.98 (2 protons; doublet; *J*, 7 cy/sec; $C{=}CHCH_2COOCH_3$), 7.88 (2 protons; distorted quartet; $CH_2C\underline{H}_2CH{=}$), 8.72 [$(CH_2)_6$], 9.11 (3 protons; distorted triplet; $C\underline{H}_3CH_2$).

From these spectra we could identify the compound as one of the two possible *cis, trans* isomers of methyl 3,5-tetradecadienoate:

$$CH_3(CH_2)_7CH{=}CH{-}CH{=}CHCH_2COOCH_3$$

Both possible isomers were synthesized. Methyl *trans*-3, *cis*-5-tetradecadienoate was synthesized from 1-decyne by the procedure reported for the synthesis of methyl 3-*trans*, 5-*cis*-*n*-tridecadienoate (*2*). The *trans*-3, *cis*-5 isomer was separated from the *cis*-3, *cis*-5 isomer by gas chromatography. The *cis*-3, *trans*-5 isomer was synthesized by the following sequence:

$$C_8H_{17}CHO + BrCH_2C{\equiv}CH \xrightarrow{Zn} C_8H_{17}CHCH_2C{\equiv}CH \quad (OH)$$

$$\xrightarrow[2.\ OH^-]{1.\ Ts\ Cl} C_8H_{17}CH{=}CHC{\equiv}CH$$

Cis and *trans* separated by gas chromatography

$$C_8H_{17}C(H){=}CC{\equiv}CH \xrightarrow[2.\ \text{ethylene oxide}]{1.\ MeMgBr} C_8H_{17}C(H){=}CC{\equiv}CCH_2CH_2OH$$

$$\xrightarrow[3.\ H_2/\text{Lindlar}]{1.\ CrO_3/H_2SO_4;\ 2.\ CH_2N_2} C_8H_{17}C(H){=}C(H){-}C(H){=}CCH_2COOCH_3$$

The infrared and nuclear magnetic resonance spectra of the synthesized *trans*-3, *cis*-5 isomer were congruent with those of the isolated compound; the retention times on Carbowax 20-M were identical. The infrared and nuclear magnetic resonance spectra of the synthesized *cis*-3, *trans*-5 isomer showed significant differences. Thus the structure of the attractant is:

$$CH_3(CH_2)_7C(H){=}C(H){-}C(H){=}CCH_2COOH$$

trans-3, *cis*-5-tetradecadienoic acid.

A small amount of the carboxylic acid was isolated directly from some active material that tailed into the methanol fraction on the silica-gel column. This fraction was purified by ion-exchange chromatography; the active fraction obtained gave a single spot on thin-layer chromatography. The methyl ester obtained, on treatment of this compound with diazomethane, was identical with the methyl ester isolated in the manner described. The infrared spectrum of the carboxylic acid showed the following diagnostic peaks (CS_2 solution; μ): ~ 2.8 to 4.2 (strong, broad COOH band superimposed on CH stretching), 5.83 (C=O), 6.85, 7.10, and 8.25 (C—O stretch and O—H bend), 10.18 and 10.55 (*cis, trans* conjugated system), 10.8 (weak; O—H bend), 13.90 (weak; CH_2 rock). The ultraviolet spectrum (methanol) was λ_{max}, 235 mμ; ϵ, ~ 25,000. The nuclear magnetic resonance spectrum (CCl_4; CAT-32 scans from 0 to 10; τ) was: ~ 3.5 to 5.0 (4 conjugated olefinic protons), 7.01 (2 protons; doublet; *J*, 7 cy/sec; $=CHC\underline{H}_2COOH$), 7.95 (2 protons; distorted quartet; $CH_2C\underline{H}_2$-CH=), 8.85 [$(CH_2)_6$], 9.22 (3 protons; distorted triplet; $C\underline{H}_3CH_2$).

The responses of male beetles to the carboxylic acid obtained by saponification of the synthetic and to the isolated *trans*-3, *cis*-5 methyl ester were identical; they mimicked the behavior (*1*) of males toward paper discs that had been in contact with females. Females did not respond in any of these tests.

No other fraction in the various stages of isolation elicited a response, but the level of response to the active component seemed to be slightly lower than that to the total extract of female beetles. Other active components may have been lost during the isolation.

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spectra, on a 21-103C and a 21-110 (CEC). Gas chromatography was carried out on an A90P3 and a 204 (Aerograph) stream splitter and flame-ionized detector.

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Cocoon Surrounding Desert-Dwelling Frogs

Abstract. *A cocoon formed from a single cell layer of shed* stratum corneum *may reduce water loss from the skin of desert-dwelling frogs while these aestivate in soil-filled burrows. In several Australian examples, the cocoon is a single layer of cells, and thus differs from the multilayered structure obtained from an American species,* Scaphiopus couchi.

Many terrestrial frogs avoid dehydration by burrowing (*1*, *2*). Frogs in burrows probably experience cool and moist conditions, and may remain there, except for brief visits to the surface during and immediately following rain. Mayhew (*3*) found that the spade-foot toad, *Scaphiopus couchi*, emerged from burrows after rain with fragments of skin-like material covering the back. These he suggested were remnants of a semi-impervious cocoon formed by the frog while underground. Similar cocoons are developed by a number of Australian burrowing frogs. Here we describe the structure of the intact cocoon, and assess its function as a barrier to water loss.

Cocoons have been found surrounding the frogs, *Cyclorana alboguttatus*, *C. platycephalus* and *C. australis*, *Limnodynastes spenceri*, and *Neobatrachus pictus*. In all instances the transparent cocoon completely surrounded the frog (Fig. 1); it was complete across the eyes and the cloaca, but there were small tubular inserts into the external nares. In some instances, the cocoon was sealed around the mouth.

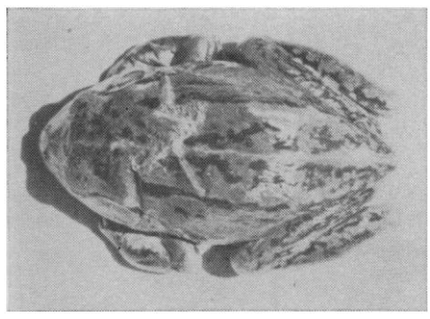

Fig. 1. Cocoon surrounding a specimen of *Cyclorana alboguttatus*.

All of the frogs with cocoons were removed from soil-filled burrows in dry clay. Cocoons surrounding frogs which had been underground for from 3 to 5 months were barely separated from the skin, and were still soft and pliable. Those from two frogs, 7 and 10 months underground, were free of the cocoon except about the head and limb extremities, and were dry but flexible. Beneath the cocoon the skin appeared moist.

Electron micrographs of sections cut tangential to the surface of the cocoon of *N. pictus* show that the cocoon is a single layer of flattened cells with the nucleus still obvious in many of the cells, and the cell membranes well-defined (Fig. 2). The intracellular spaces are filled with sheaves of fine fibrils of keratin. The intercellular contacts between the cells are well-marked, strong, and persistent. A comparison with the structure of the intact skin of the same frog (Fig. 3) shows that the cocoon is identical to the *stratum corneum* of the epidermis, and is derived by sloughing this layer as a compact unit, as occurs in snakes. The separation occurs intercellularly; the contents of numerous small vesicles, which gather beneath the cell membranes before separation and are absent afterwards, appear to enter the intercellular space and may facilitate separation. These cocoons appear to differ from that described for *Scaphiopus couchi*, where there is a keratinization and darkening of the skin after 1 month underground (*4*) with the formation of a black membrane of several layers (*3*).

The rate of passage of water vapor through cocoons of *C. alboguttatus* and *N. pictus* was measured by placing pieces of a cocoon between a saturated atmosphere (against the surface proximal to the frog) and silica gel, both at 25°C. The weight gained by the silica gel represented the weight of water absorbed through the membrane. For comparison, rates of dehydration of four *N. pictus* and eight *C. alboguttatus* were obtained under identical conditions. As activity influenced the weight lost by the frogs, only those hourly periods during which the frogs remained still were used to estimate the rate of dehydration.

The average rate of passage of water vapor through the cocoons of eight *C. alboguttatus* was 0.65 (S.D. ± 0.22) mg of water per square centimeter per hour and through a single cocoon from *N. pictus* it was 0.51 mg. These values are similar, and both are significantly lower ($P < .01$) than the average rates of water loss [expressed in terms of body surface where $S = 6^3\sqrt{(W)^2}$ (*5*)] from frogs of the same species in an identical atmosphere. In eight *C. alboguttatus*, the rate of water loss from the whole frog was 4.90 (± 0.08) mg cm^{-2} hr^{-1} and for four *N. pictus* it was 9.0 (± 0.10) mg. However, a direct comparison with cutaneous water loss is not possible since water is lost from the lungs as well as the skin in these frogs.

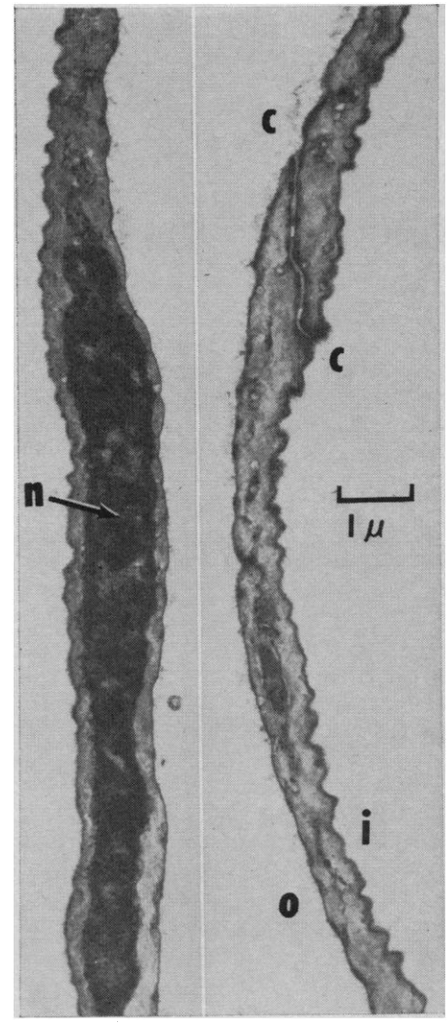

Fig. 2. Electron micrographs of a cross section of two portions of a fold in a cocoon from *Neobatrachus pictus*. The material was fixed in osmium fixative and stained with uranyl acetate and a lead preparation. Details of cellular structure are still evident: *n*, nuclear remnant; *o*, outer cellular surface; *i*, inner cellular surface; *c*, persistent intercellular contact.