

Fig. 2. Identification of 2-nonenal (retention time 50.57 min) by GLC of the atmospheric steam distilled oil of the carrot cultivar Regol. Operating conditions: instrument, Hewlett Packard 5750G equipped with automatic computing integrator 3380A; column, glass 3 m×4 mm inner diameter; packing, 7% Carbowax 20 M; injection port temperature, isothermal at 60°C for 20 min followed by a programmed 2°C/min, rise to 170°C. Identification is by comparison with the retention time of trans-2-nonenal and quantification is by reference to known amounts of menthol using the internal standard method.

the compound in the oil for collection and confirmation by other methods of its isomeric form. Nevertheless, taken in conjunction with the earlier reports<sup>5,6</sup> it very strongly suggests that 2-nonenal is present in the oil and the trans form is most likely due to its greater stability.

This is not the only indication of a protective role for 2-nonenal, as it has been identified in the defensive secretion

of the tenebrionid beetle *Eleodes beameri* (Blais)<sup>7</sup>, but it seems the first demonstration of it as an insecticide.

The existence of a plant-associated compound with such a profound effect on its parasite is consistent with current views on the co-evolution of plants and insects<sup>3</sup> and investigation of its field efficacy and mode of action against *P. rosae* would seem appropriate.

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## Physico-chemical properties of South American iguanid albumins

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**Summary.** Except for a markedly reduced anodal electrophoretic mobility, the serum albumin of the Galapagos marine iguana was physico-chemically identical to that of terrestrial iguanids. Reduction in albumin net charge may have facilitated the adaptation of this species to a semi-aquatic environment.

Among the Reptilia, plasma osmotic pressures directly relate to albumin concentration<sup>1</sup>. Both the charge and plasma levels of this protein vary throughout the range of reptilian species, apparently to accommodate specific environmental conditions<sup>1</sup>. This adaptability appears to have been a major factor in the shaping of reptilian evolution.

Serological differences exist among the serum albumins of several closely related genera of Galapagos land and marine iguanas<sup>2</sup>. Since these reptiles occupy distinct habitats within the Galapagos region, such variations may reflect particular biochemical adaptations to their respective environments. In order to further probe the relationship between environment and serum protein modifications, the physical properties of several South American iguanid albumins were analyzed; the results form the basis of this report. These observations include the first compara-

tive biophysical study of Galapagos iguana serum albumins.

**Materials and methods.** Adult iguanas, *Amblyrhynchus cristatus* (Galapagos marine iguana), *Conolophus pallidus* and *C. subcristatus* (Galapagos land iguanas), and *Iguana iguana* (mainland South American iguana), were bled by cardiac puncture and the sera separated from clotted blood by centrifugation<sup>3</sup>. Serum albumins were isolated by precipitation in 10% trichloroacetic acid followed by solubilization with 100% ethanol<sup>2</sup>. Electrophoresis of whole sera and purified albumins utilized cellulose acetate/Tris-barbital pH 8.8<sup>4</sup> and 7% acrylamide/Tris-glycine pH 9.2<sup>2</sup> systems. Molecular sieve chromatography of iguana serum proteins was done on Sephadex G-200 (Pharmacia) with 0.15 M NaCl/10<sup>-3</sup> M Tris buffer, pH 6.8; absorbancy of 2-ml effluent fractions was recorded at 280 nm. Albumin molec-

ular weight was determined by electrophoresis in 7.5% acrylamide/Tris-sodium dodecyl sulfate buffer pH 8.8<sup>5</sup>. The sedimentation coefficient of iguana albumins was measured by velocity centrifugation in 10–30% (v/v) glycerol gradients using 0.2 M potassium phosphate buffer pH 8.0<sup>6</sup>; monomeric bovine serum albumin served as a reference standard. Evaluations of albumin heterogeneity utilized diethylaminoethyl (DEAE)-Sephadex A-50 ion-exchange chromatography<sup>2</sup>; albumin solubilities were assayed in aqueous solutions of ammonium sulfate. Albumin was identified in column effluents and ammonium sulfate

Physical characteristics of iguanid serum albumins

Property	Species <i>A. cri-</i> <i>status</i>	<i>C. pal-</i> <i>lidus</i>	<i>C. subcri-</i> <i>status</i>	<i>I. iguana</i>
Anodal mobility <sup>a</sup>	0.93	1.22	1.17	1.11
Anodal mobility <sup>b</sup>	0.86	1.08	1.04	1.05
Molecular weight <sup>c</sup>	69 × 10 <sup>3</sup>	69 × 10 <sup>3</sup>	69 × 10 <sup>3</sup>	69 × 10 <sup>3</sup>
Ve/Vo <sup>d</sup>	2.2	2.3	2.4	2.4
S <sub>20,w</sub> × 10 <sup>13</sup> <sup>e</sup>	4.5	4.5	4.5	4.5
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> Sol. <sup>f</sup>	2.6	2.6	2.6	2.6
A-50 elution <sup>g</sup>	1	1	1	1
% serum protein <sup>h</sup>	36–41	36–38	35–39	42–44
Polymer formation <sup>i</sup>	yes	yes	yes	yes

<sup>a</sup> Relative to human albumin; cellulose acetate. <sup>b</sup> Relative to mouse albumin; 7% acrylamide gel. <sup>c</sup> SDS-acrylamide gel electrophoresis. <sup>d</sup> Elution volume/column void volume; Sephadex G-200 chromatography. <sup>e</sup> Sedimentation coefficient. <sup>f</sup> Molarity of ammonium sulfate at which albumin consistently remained in the supernatant fraction. <sup>g</sup> Determination of heterogeneity; number of peaks of anti-albumin reactive protein resolved upon DEAE-Sephadex A-50 ion-exchange chromatography of trichloroacetic acid-precipitated ethanol-soluble iguana serum albumins. <sup>h</sup> Albumin contribution to total serum protein as estimated by densitometry of amidoblack stained polyacrylamide separations of whole sera. <sup>i</sup> Tendency to form dimers and higher polymers upon storage; Sephadex G-200 chromatography of albumins maintained at –20°C for 3 months.

fractions by agar double-diffusion assay using rabbit anti-serum to iguana albumin<sup>2</sup>.

**Results and discussion.** Except for a markedly reduced anodal electrophoretic mobility, the albumin of the Galapagos marine iguana resembled, both in concentration and physical properties, that of terrestrial iguanid species (table). The albumin level in serum, as estimated by gel densitometry of stained acrylamide gel separations, was similar for each of the genera examined and ranged from 35 to 44% of the total serum protein. The accuracy of this method for estimation of albumin concentration is evidenced by the similarity of values observed here and those reported for *Iguana* using different methodologies<sup>7</sup>. Both estimates place the albumin contribution at 42–44% of the total *Iguana* serum protein.

A relatively slow anodal electrophoretic mobility, indicative of low net charge, is the only significant biophysical property differentiating the serum albumin of the marine iguana from that of terrestrial iguanids. Charge reduction on this protein, therefore, may be critical to successful reptilian adaptation to an aquatic environment. In this respect, marine iguana serum albumin resembles the low charge density albumin-like protein of certain fresh-water chelonians<sup>1</sup>.

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#### Antiparasitic agents. 4. Injectable phenylguanidine anthelmintics<sup>1</sup>

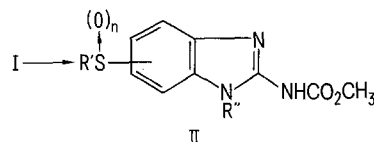
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**Summary.** A series of phenylguanidine anthelmintics has been discovered to be active by injection against nematode and trematode species.

Earlier we reported on injectable benzimidazole anthelmintics which were effective against nematode, cestode and trematode species<sup>2</sup>. Similar but somewhat reduced activity has been discovered for phenylguanidines of Structure I, which can be regarded as prodrugs of the corresponding benzimidazoles II. Some typical representatives of this series are listed in the table. The synthesis of this class of compounds is exemplified by the preparation of 7. 4-Amino-3-nitrothiophenol (Na-salt), prepared by sodium borohydride reduction of 4-thiocyano-2-nitroaniline, is alkylated with isobutyl chloride to furnish the corresponding sulfide. Acylation of the sulfide with methylthioacetyl chloride yields the acetamide. Catalytic hydrogenation of this material and subsequent reaction of the resulting aniline with 1,3-bis-(methoxycarbonyl)-5-methylthiourea yields [[2-[[[(Methylthio)acetyl]amino]-4-[(2-methylpropyl)-thio]amino]](methoxycarbonyl)amino]methylene] carbamic

acid, methyl ester. Oxidation with m-chloroperbenzoic acid affords the disulfoxide 7.



A single s.c. injection of **1** (suspended in water) at 5 and 10 mg/kg to naturally infected sheep eliminated approximately 80 and 95% respectively of *Haemonchus*, *Ostertagia*, *Trichostrongylus* in the abomasum, and *Nematodirus*, *Trichostrongylus*, *Oesophagostomum* and *Chabertia* in the small and large intestines. A single oral dose of **1** at 10 mg/kg eliminated approximately 99% of the above listed genera. Compound **1** was not effective in sheep naturally infected with tapeworms of the genus *Moniezia* when