

Bile Acid Composition of Some Lizards from Southwestern United States¹ (39594)I. M. YOUSEF,² W. G. BRADLEY,³ AND M. K. YOUSEF⁴

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Haselwood suggested a relationship between the chemical types of bile acid and the systematic classification of vertebrates (1). Our knowledge of bile acid composition in different vertebrates is limited to only a few species and families, and more comparative data are needed at species, generic, and family levels. Only after the analysis of such data can realistic and worthwhile generalizations be developed concerning possible evolutionary patterns for bile acids in vertebrates.

Lizards are both a phylogenetically diverse and an ancient group which is found throughout much of the world in a wide array of ecologic roles and habitats (2). Studies of bile acid composition in this group would greatly aid in our understanding of the relationship of chemical structure of bile acids and their evolution within vertebrates. As an initial stage in such an investigation we have identified the bile acids from nine species of lizards all common in southwestern United States. Two lizard families, Gekkonidae and Teiidae, are represented by a single species, while the ecologically diverse Iguanidae is represented by seven species. This allows for comparisons of bile acid composition between three families, between genera within a family, between species of the same genus, and between species which differ in diet and habitat.

Materials and methods. Bile was obtained

from the gallbladders of 31 lizards of nine different species from southern Nevada. Lizards were collected alive and sacrificed within 3 days of capture. The gallbladders were removed intact and frozen until analysis.

The bile (0.2–1.0 ml) was extracted by the addition of 10 ml of hot ethanol-methanol and shaking (3). The precipitated protein was removed by centrifugation at 2000g at 4° for 15 min. The alcoholic extract was decanted, and the precipitate was washed with 2 ml of hot ethanol-methanol and filtered. The extracts were combined, were diluted to 40% alcohol with distilled water, and were delipidated with petroleum ether. The aqueous alcohol solution was then evaporated to dryness under nitrogen *in vacuo*. The residue was dissolved in 1 ml of methanol.

An aliquot of the bile acid extract was applied as a spot to a tlc plate (20 × 20 cm) coated with a silica gel G (Merck & Co.) in a 0.25-mm thick layer. The plates were developed in *n*-butanol-glacial acetic acid-water (10:1:1, v/v for 4 hr) (4). After evaporation of the solvents, the bile acids were located by iodine vapor, and relative R_f values of any bands were compared to known standards of free cholic acid (CA), chenodeoxycholic acid (CDCA), and their taurine and glycine conjugate for identification. The rest of the bile extract was hydrolyzed at room temperature (24 hr) with acetone-ethanol (9:1) adjusted to pH 1.0 with 2 *N* HCl (5). The solvents were then evaporated and subjected to alkaline hydrolysis as previously described, and free bile acids were extracted (3). Bile acids were measured by gas-liquid chromatography (glc) and for this purpose the bile acids were converted into methyl esters by methanol 2,2-dimethoxy-propane (Aldrich Chemical Co., Milwaukee, Wisc.) and concentrated HCl (6). Prior to glc, the

¹ Supported in part by NSF Grant No. GB 35281 and MRC Grant No. MA 4865.

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methyl esters were converted into trifluoroacetate by reaction with 0.3 ml of trifluoroacetic anhydride at 40° for 40 min. The glc analyses were performed on a Packard 7401 gas chromatograph system equipped with dual glass columns (1.2-m × 2-mm i.d.) containing 3% OV-210 on 80–100 mesh gas Chromosorb W (Chromatographic Specialties, Ontario). The separation was made at 225° isothermally using helium (30 ml/min) as the carrier gas. The glc system was calibrated by means of standard mixture previously described (3). The known bile acids methyl ester were quantitated using 5β-cholanic acid as internal standard (3).

The samples were further analyzed on combined gas-liquid chromatography-mass spectrometry (glc-ms) Varian Mat CH-5 single-focusing mass spectrometer coupled to a Varian Mat computer as previously described (6).

Bile acid percentages were compared between families and between the seven iguanid species using a similarity index (IS) described previously (7):

$$IS = \frac{2M_w}{MA + MB} \times 100,$$

where M_w refers to the sum of the smaller quantitative values common to two samples, MA is the sum of the quantitative values for one sample, and MB is the sum of the values for the other sample. Since both samples each have a total quantitative value of 100, the formula can be rewritten as:

$$IS = \Sigma M_w.$$

Results. The tlc of the conjugated bile acids of the nine species studied showed only tauro-bile acids, and no traces of sulfate, glycine-conjugated bile acids, or free bile acids could be detected. The glc of the species studied is shown in Fig. 1. It is apparent that most of the species studied contained at least five peaks which were further identified by glc-ms as allocholic acid (AC), cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DOCA), and lithocholic acid (LCA). The molar percentage of each bile acid varied from species to species.

The average molar percentage of different bile acids for each species is shown in

Table I. In Gakkonidae and Teiidae, CA was 70 and 44%, respectively, as compared to 2–13% in the seven species of Iguanidae. The seven species of iguanids studied all exhibited high percentages of AC (64–91%) as compared to 6% in the other two families. Only trace amounts of LCA were detected in all species except *Cnemidophorus*. In *Cnemidophorus* an additional bile acid, representing 6% of the total, was not identified.

The bile acid spectra for the three lizard families are further analyzed in Table II, where IS values for bile acid percentages shared between families were calculated (7). These indices provide a method for determining the degree of similarity in the total pattern of bile acids for different groups. Bile acid spectra of Gekkonidae and Teiidae showed a high IS as compared with Iguanidae. Similarly, bile acid spectra of the seven species of iguanids are compared in Table III. Similarity indices were consistently high ranging from 73 to 97%.

Discussion. Lizard bile acids have been reported for only a few species. Haslewood (1) in two species of *Anolis* (Iguanidae) reported the presence of AC and CA, but recently Okuda *et al.* (10) studied the bile acid of *Iguana iguana* (Iguanidae) and reported the presence of AC as the major bile acid and 3α-, 7α-, 12α-trihydroxy-5α-cholestan-26-oic acid as the second major bile acid in this species, but CA was not detected. Their finding was supported by the lack of enzymatic activity to reduce 7α-hydroxycholest-4-en-3-one to 5β alcohol in the liver as reported by others (11).

In our study, AC and CA were found in all species studied (Table I). Among the iguanids studied, AC was the primary bile acid which is compatible with findings for *Anolis* and for *Iguana iguana* (1, 10). Evidently, the molar percentages of AC and CA are variable among iguanids and should be studied further, particularly for more species. Of considerable importance is the low concentration of AC in the single species studied in both the Teiidae and Gekkonidae. Also, CDCA, DOCA, and LCA were found in varying amounts in all but one of the species studied.

Reptiles are an ancient and largely extinct

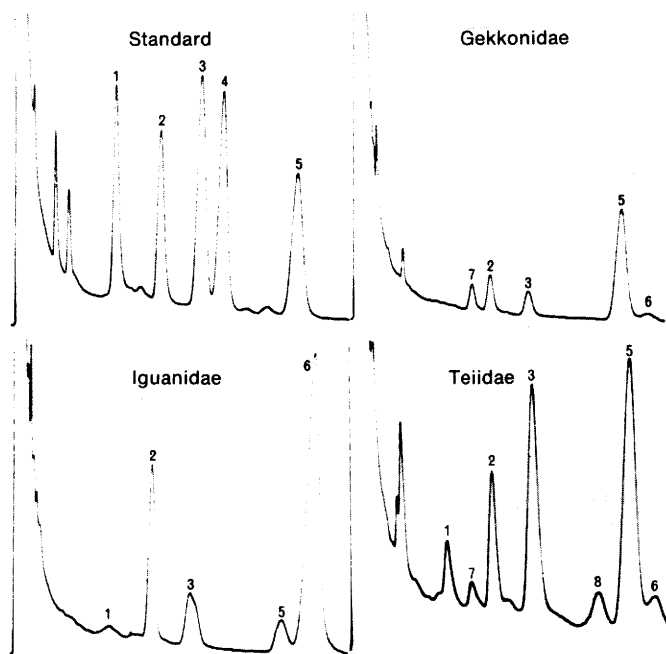


FIG. 1. A typical gas chromatogram of trifluoroacetates of bile acid methyl esters. Column 3% OV-210 on 80-100 mesh Chromosorb W operating temperatures were: Column 225°, injection 225°, and detection 240°. The carrier gas was helium and its flow was 37.5 cc/min. Chart speed 5 min/2.5 cm. The numbers 1 to 8 represent the following: 1, lithocholic acid; 2, deoxycholic acid; 3, chenodeoxycholic acid; 4, hydoxycholic acid; 5, cholic acid; 6, allocholic acid; 7, unknown; 8, unknown.

TABLE I. CLASSIFICATION, SAMPLE SIZE, BODY WEIGHT, AND MOLAR PERCENTAGE OF DIFFERENT BILE ACIDS FROM BILE OF LIZARDS.

Classification and common name	Sample size	Av body wt (g)	Allocholic acid (AC)	Cholic acid (CA)	Chenodeoxycholic acid (CDCA)	Deoxycholic acid (DOCA)	Lithocholic acid (LCA)	Other
Infraorder Gekkota								
Family Gekkonidae								
<i>Coleonyx variegatus</i> (Western banded gecko)	4	2.2	5.7	69.5	10.5	14.2	T ^b	T ^b
Infraorder Iguania								
Family Iguanidae								
<i>Dipsosaurus dorsalis</i> (Desert crested lizard)	7	44.0	85.0	2.1	— ^a	12.9	T ^b	— ^a
<i>Sceloporus magister</i> (Desert spiny lizard)	3	40.0	63.7	6.7	7.3	22.3	T ^b	— ^a
<i>Sceloporus occidentalis</i> (Western fence lizard)	7	16.0	85.9	10.1	1.3	2.8	T ^b	— ^a
<i>Sceloporus graciosus</i> (Sagebrush lizard)	1	6.0	84.6	8.7	2.1	4.6	T ^b	— ^a
<i>Urosaurus graciosus</i> (Long-tailed brush lizard)	1	4.0	90.0	5.0	5.0	T ^b	T ^b	— ^a
<i>Uta stansburiana</i> (Side-blotched lizard)	2	3.0	75.9	12.9	6.1	5.1	T ^b	— ^a
<i>Phrynosoma platyrhinos</i> (Desert horned lizard)	5	24.0	91.0	1.6	1.0	6.4	T ^b	— ^a
Infraorder Scincomorpha								
Family Teiidae								
<i>Cnemidophorus tigris</i> (Western whiptail)	2	11.5	6.0	44.4	25.8	14.2	3.5	6.0

^a —, Is not detectable.

^b Trace is less than 0.1%.

group with snakes and lizards making up the bulk of the recent reptile fauna (2). Lizards, especially, are almost worldwide in distribution, with the exception of arctic and antarctic regions. The three families studied are not closely related phylogenetically, being found in three separate infraorders. The Gekkonidae are primarily an old world group with related families of Jurassic origin. Some species are present on all continents except Antarctica. Fossils from families related to the Iguanidae are also of Jurassic origin. This family, with the exception of two fossil genera, is largely restricted to the new world. The Teiidae is a group of new world lizards which date back to Cretaceous time. Therefore, the three lizard families studied all evolved during the Mesozoic with the Gekkonidae of old world origin, and both Iguanidae and Teiidae of either new world origin or major affinity.

It is surprising that the IS between Gekkonidae and Teiidae is so high (75%), considering that they are distantly related phylogenetically and had their major evolution in old and new world, respectively. In contrast, the Iguanidae have extremely low IS values when compared with either group. Speculation on possible evolutionary relationships based upon bile acid compositions should be curtailed, however, until further study of other species of teiids and gekkonids are conducted.

Similarity indices for the seven species of iguanids are surprisingly high. In general, IS values for species of rodents belonging to

the same family are considerably lower (12). Within the three species of *Sceloporus* studied, IS values were lower in one instance than most comparisons between genera. In part, the high IS values are due to the high percentages of AC found in all species which allow for only minor differences in the percentages of the other bile acids.

The portion of various bile acids appear to be little affected by diet as the single vegetarian, *Dipsosaurus dorsalis*, has comparable IS values with the insectivorous species. The nine species studied occur in a wide variety of habitats in the Mohave Desert (13). There appears to be no apparent relationship between ecologic distribution and IS values.

To our knowledge this is the first study concerning the quantitative distribution of bile acids in a large group of lizard species occurring in the same geographic region. It represents a start in our understanding of bile acid relationships among lizards. We plan further studies involving more families, genera, and species, which will hopefully allow meaningful analysis of evolutionary and biochemical relationships.

Summary. Nine species of lizards representing three families were used to investigate bile acid composition of the bile acid pool isolated from gallbladders. Members of the three families contained AC, CA, CDCA, DOCA, and LCA in varying proportions. Gekkonids and teiids, represented by a single species each, had quite similar bile acid spectra, with CA as the primary bile acid. This is surprising since these families have different geographic origins and are only distantly phylogenetically related. In contrast, the seven species of iguanids had AC as the primary bile acid. At both generic and species levels, the iguanids studied had quite similar bile acid spectra.

TABLE II. BILE ACID SIMILARITY INDICES FOR LIZARD FAMILIES

	Teiidae	Iguanidae ^a
Gekkonidae	75	23
Teiidae		24

^a Bile acid percentages for the seven iguanid species were averaged.

TABLE III. BILE ACID SIMILARITY INDICES FOR SEVEN SPECIES OF IGUANID LIZARDS.

	<i>Sceloporus occidentalis</i>	<i>Sceloporus graciosus</i>	<i>Uta stansburiana</i>	<i>Dipsosaurus dorsalis</i>	<i>Urosaurus graciosus</i>	<i>Phrynosoma platyrhinos</i>
<i>Sceloporus magister</i>	75	77	82	79	74	73
<i>Sceloporus occidentalis</i>		97	90	90	92	91
<i>Sceloporus graciosus</i>			91	91	92	92
<i>Uta stansburiana</i>				83	86	84
<i>Dipsosaurus dorsalis</i>					87	93
<i>Urosaurus graciosus</i>						93

Within this group, food habits or ecologic distribution appeared to have little effect on the proportion of bile acids.

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Received October 22, 1974. P.S.E.B.M. 1977, Vol. 154.