DISCUSSION

The results obtained show that, as with growing cultures of the yeast, the amounts of ester formed by washed suspensions are too large to be accounted for by the reversal of a simple esterase reaction, indicating a more exergonic mechanism of ester formation. This is confirmed by the fact that, under anaerobic conditions, no ester is formed from ethanol plus acetate or from acetaldehyde, showing that oxidation is necessary for the synthesis of ester.

In the case of ester formation from (ethanol + acetate), there is no evidence that the acid moiety of the ester is derived from the added acetate. The marked stimulation of ester production followed by inhibition, occurring when the acetate concentration is increased at pH 4·6, may be due to an alteration of the external pH required to give optimal internal conditions for ester formation from ethanol alone. This explanation would also account for the effect of increasing the acetate concentration at pH 2·8 and the behaviour in presence of different buffers. The problem of the origin of the acetate moiety of the ester could be readily investigated by suitable isotope experiments.

It is possible that oxidation of ethanol may lead to the formation of a labile acetyl derivative (cf. Stadtman & Barker, 1949; Black, 1950) which then reacts with further ethanol to produce the ester. This hypothesis can only be tested by investigation of ester synthesis in a cell-free system. Attempts to prepare such a system are being made.

SUMMARY

1. Under aerobic conditions, washed suspensions of *Hansenula anomala* form ester from ethanol, alone or in presence of acetate.

2. No ester is formed in the absence of oxygen.

3. The ester formed is ethyl acetate.

4. The ester formed disappears after exhaustion of the ethanol substrate.

5. The effects of pH, age of culture, ethanol concentration and acetate concentration on ester formation have been investigated.

6. A number of alcohols have been tested but ester is formed at an appreciable rate only from ethanol or glucose. Acetaldehyde does not give rise to any ester, but in low concentrations acetaldehyde stimulates ester formation in presence of ethanol.

I wish to express thanks to my colleagues, in particular to Dr R. Davies and Dr E. F. Gale, for advice, criticism and encouragement during the course of this work. My thanks are also due to the Medical Research Council for a training grant, and to Trinity College, Cambridge, for an exhibition.

REFERENCES

Black, S. (1950). *Fed. Proc.* **9**, 152.

Clark, W. M. (1948). *The determination of hydrogen ions.* London: Baillière, Tindall and Cox.

Davies, R., Falkiner, E. A., Wilkinson, J. F. & Peel, J. L. (1951). *Biochem. J.* **49**, 58.

Elsden, S. R. (1946). *Biochem. J.* **40**, 252.

Hestrin, S. (1949). *J. biol. Chem.* **180**, 249.

Keenan, A. G. (1945). *Canad. chem. J.* **29**, 857.

Lipmann, F. & Tuttle, L. C. (1945). *J. biol. Chem.* **159**, 21.

Mitchell, P. D. (1949). *Nature, Lond.*, **164**, 846.

Northrop, J. H., Ashe, L. H. & Senior, K. S. (1919). *J. biol. Chem.* **39**, 1.

Parks, G. S. & Huffman, H. M. (1932). *Free Energies of some Organic Compounds.* New York: Chemical Catalog Co.

Stadtman, E. R. & Barker, H. A. (1949). *J. biol. Chem.* **180**, 1095.

Comparative Studies of 'Bile Salts'

2. PYTHOCHOLIC ACID

By G. A. D. HASLEWOOD and VERYAN M. WOOTTON
Guy's Hospital Medical School, London, S.E. 1

(*Received* 10 *November* 1950)

It was shown in Part 1 (Haslewood & Wootton, 1950*b*) that the bile of three species of Boidae contained a new acid, $C_{24}H_{40}O_5$, then called by us pythocholic acid, which was easily converted into pythocholic lactone, $C_{24}H_{38}O_4$. In the African python at least, this acid, conjugated with taurine, formed the main constituent of the bile salts.

The work now described had two aims; to explore further how far pythocholic acid is characteristic of the bile of the Boidae, and to elucidate as far as possible the chemistry of the new acid.

A preliminary communication on this work has appeared (Haslewood & Wootton, 1950*a*).

RESULTS

Biological. Approximate figures for the content of pythocholic acid in the total (hydrolysed) 'bile acids' from the bile of seven species of Boidae are

Table 1. *Bile acids of Boidae*

(Approximate amounts of cholic and pythocholic acids in the 'bile acids' (see text, p. 69) of seven species of Boidae. Based on the isolation of crystalline material (methyl pythocholate, pythocholic lactone, cholic acid). Classification of snakes after Smith, 1927.)

Species	Pythocholic acid (percentage of 'bile acids'; approx. minimal figures)	Cholic acid (Hammarsten test, or approx. percentage of 'bile acids')
Green tree boa, *Boa canina*	28	3
Common boa, *Constrictor constrictor*	51	H-test, negative
Emperor boa, *C. imperator*	54	H-test, negative
Western boa, *C. occidentalis*	52	4
Rainbow boa, *Epicrates cenchris*	53	H-test, ?negative
Indian python, *Python molurus*	70	H-test, negative
African python, *P. sebae*	70	H-test, negative

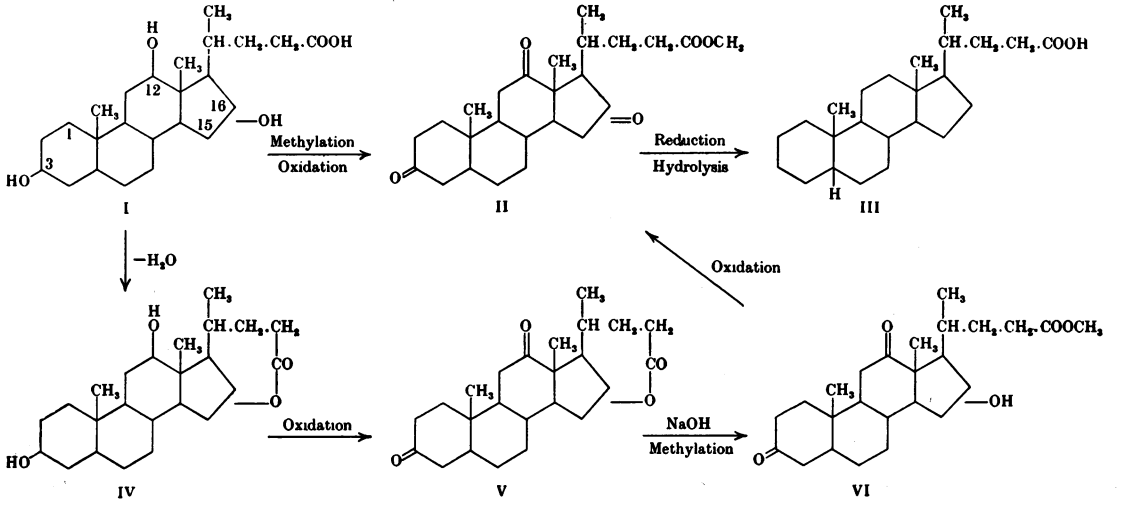
given in Table 1, together with an indication of the presence or absence of cholic acid in these biles. Cholic acid was isolated from the bile of two species: a positive Hammarsten test thus probably indicates its presence in the bile of other Boidae.

Chemical. Results of the experiments described in full below can be interpreted as follows.

Pythocholic acid $C_{24}H_{40}O_5$ (I) formed a methyl ester which was oxidized by chromic oxide to the triketone methyl dehydropythocholate (II); and this was reduced by the Kishner-Wolff method to cholanic acid (III). Hence pythocholic acid contains three secondary hydroxyl groups attached to the cholanic acid nucleus.

Pythocholic lactone (IV) formed a diketone, dehydropythocholic lactone (V) on chromic oxidation. It also gave a diacetyl derivative, which could be recovered unchanged after treatment with chromic oxide, and a monoacetate, the free OH group of which was oxidized to give a monoketone, characterized by a semicarbazone. This behaviour, together with the fact that pythocholic acid does not respond to the Hammarsten test, strongly suggests that one of the two hydroxyl groups in pythocholic lactone is at C_{12}; on biogenetic grounds the other may be at C_3. This suggestion is not contradicted by the optical findings, for dehydrogenation of hydroxyl groups at C_3 and C_{12} in deoxycholic acid produced an increase in molecular rotation of roughly the same amount as was found when pythocholic lactone was converted to the dehydro-compound. $[M]_D$3:12-diketocholanic acid (dehydrodeoxycholic acid) $-$ $[M]_D$3α;12α-dihydroxycholanic acid (deoxycholic acid) $= 349° - 153° = +196°$. $[M]_D$ dehydropythocholic lactone $-$ $[M]_D$ pythocholic lactone $= 448° - 203° = +245°$. 3:11-Dihydroxy compounds show a small rotation change on oxidation to diketones, while 3:6- and 3:7-dihydroxy compounds show considerable negative rotation changes (for summary of data, see Barton & Klyne, 1948).

In the conditions used, opening of the lactone ring in dehydropythocholic lactone (V), followed by methylation, led to molecular changes; in one experiment it was possible to isolate, in small yield, an impure compound, probably (VI), which was converted to methyl dehydropythocholate (II) and thence to cholanic acid (III). In another case, however, the crystalline intermediate compound could not be converted to methyl dehydropythocholate; it was apparently an isomer of (VI).



Partial oxidation of methyl pythocholate followed by Kishner-Wolff reduction of the product gave a mixture from which was isolated an acid, apparently one of the expected deoxypythocholic acids, $C_{24}H_{40}O_4$, formed by elimination of a hydroxyl group at C_3 or C_{12} from pythocholic acid.

On the assumption that two OH groups in pythocholic acid are at C_3 and C_{12}, and by comparison with methyl deoxycholate ($[M]_D + 195°$; Charonnat & Gauthier, 1947), it can be calculated that the molecular rotation difference due to inclusion of the lactone-forming OH group in methyl pythocholate is approximately $-72°$. Dehydrogenation of this group produced the $[M]_D$ difference of $-341°$, derived as follows:

	$[M]_D$
Dehydrogenation of two OH groups not concerned in lactonization (see below)	+245°
Methyl dehydropythocholate	− 96°
Change due to dehydrogenation of lactone-forming OH group	−341°

EXPERIMENTAL

General. Melting points are uncorrected. Optical rotations were determined in a 1 dm. micro-tube. Micro-analyses (C and H) were done by Drs Weiler and Strauss, Oxford. Al_2O_3 (Hopkin and Williams) was neutralized and reactivated (Shoppee, 1949). L.p. = light petroleum, b.p. 40–60°. 20% CrO_3 = a solution made by dissolving 20 g. of CrO_3 in the minimum amount of water and making to 100 ml. with acetic acid. H-test = Hammarsten's HCl test (Haslewood, 1943).

Isolation of acids

Methyl pythocholate from bile. The bile salts (0·5–1 g.) were obtained and hydrolysed as previously described (Haslewood & Wootton, 1950*b*). The 'bile acids', precipitated with HCl and NaCl (excess) were collected at once, washed with water and dried *in vacuo* over H_2SO_4. The mother liquors in all cases were tested with $BaCl_2$ for SO_4^{--}; only traces of this ion were detected. After weighing and examination by the H-test, the dried 'bile acids' were dissolved in ethanol and this was saturated with diazomethane, carried over in N_2 from nitrosomethylurea decomposed in the usual way. The ethanolic solution was acidified with H_2SO_4, diluted with water and extracted with ether. The ether was washed with water, dil. NH_3, water, dried (Na_2SO_4) and evaporated. The residue, from the bile of all the species mentioned in Table 1, crystallized at once; it was collected, dried and weighed. After recrystallization from dilute ethanol, white needles of a hydrate were obtained. After drying at about 80° *methyl pythocholate* had m.p. 146–148°; $[\alpha]_D^{23°} = +28° \pm 1°$ in $CHCl_3$ (*c*, 1·2). $[M]_D = +123°$. (Found: C, 68·4; H, 10·4. $C_{25}H_{42}O_5$, H_2O requires C, 68·2; H, 10·0%.)

The esters obtained from six of the species of Boidae examined were identical in melting point. Mixed melting points with the esters from the African python (taken as standard) gave no depression. Yields of crude crystalline ester and/or lactone (see below) and results of the H-test are given in Table 1. Methyl pythocholate was sparingly soluble in ether and dissolved readily in $CHCl_3$ and ethanol; all crystals obtained appeared to be hydrated and from wet ether or dilute ethanol often had, before drying at about 80°, a substantially lower m.p. (approx. 115 or 135°, with effervescence) than that quoted above. Owing to the slight solubility in ether and ease of crystal formation of hydrated methyl pythocholate, the above method appeared to provide an approximately quantitative measure of the pythocholic acid in the bile salts.

In our earlier experiments, we relied on the isolation of the crystalline lactone from ethyl acetate, after preliminary warming of the crude 'bile salts' with dil. HCl. However, further work showed that the yield of lactone was very variable, depending more on the boiling of crude pythocholic acid with ethyl acetate than on allowing it to stand in acidified solutions. There seems to be no doubt that our earlier separation of ethyl esters from the bile salts of *Constrictor occidentalis* and *Python molurus* on Al_2O_3 columns led only to samples of crude ethyl pythocholate and pythocholic acid. Methyl and ethyl pythocholate appear to be easily hydrolysed on Al_2O_3 (see below).

Hydrolysis of purified methyl pythocholate (50 mg.) in ethanol (0·5 ml.) with 40% (w/v) KOH (2 drops) at 70° for 10 min. gave, after acidification, pythocholic acid which crystallized from ethyl acetate in large colourless prisms (20 mg.) having m.p. 186–187° when heated rapidly; the melting point was higher and indefinite with slow heating, presumably because of lactone formation.

Cholic acid from Constrictor occidentalis. A benzene solution of 0·306 g. of ethylated 'bile acids', prepared as described in Part 1, was poured on to a column of Al_2O_3 (3 g.). About half the material was hydrolysed on the column. 80 mg., eluted with benzene and ether/benzene, gave a negative H-test; 11 mg. eluted with ether, and 60 mg. eluted with ethanol showed a strongly positive response to this test. The latter material (60 mg.) would not crystallize, but on hydrolysis it gave, from ethyl acetate, a crystalline acid (10 mg., about 3·3% of the total 'bile acids') which, after recrystallization from ethyl acetate, formed typical prisms (5 mg., H-test, blue) of cholic acid m.p. 193–195°, not depressed by an authentic sample.

Table 2. *Separation of methyl esters from the 'bile acids' of Boa canina*

(339 mg. of esters, left from the crystallization of methyl pythocholate, on 4 g. Al_2O_3.)

Fraction	Eluent (ml. solvent)	Wt. (mg.) etc.
I	30 benzene	161 crystallized with ether H-test, negative
II	25 benzene	13·6 H-test, positive
III	50 benzene	12·3 H-test, positive
IV	25 ether	79·0 H-test, positive
V	25 ether	38·5 H-test, positive
VI	25 ether	23·3 H-test, positive

Cholic acid from Boa canina. Methyl pythocholate (0·15 g.) was crystallized from an ether solution of the methyl esters of the 'bile acids' (0·598 g.) from the bile salts (0·935 g., from one gall bladder) of this species. The mother liquors were evaporated. The residue (0·339 g.) was dissolved in benzene and poured on to Al_2O_3 (4 g.) in a wide column which was rapidly eluted as described in Table 2. Perhaps, because of rapid elution, hydrolysis was negligible. Fraction I, with ether, gave 20 mg. of pythocholic lactone. Fractions IV–VI

were combined and hydrolysed by heating in ethanol (1·5 ml.) with 40% (w/v) KOH (0·2 ml.) at about 80° for 20 min. The diluted solution was acidified with HCl and saturated with NaCl. The gummy precipitate was collected, washed and crystallized from ethyl acetate, from which cholic acid (15 mg., H-test, blue; about 2·5% of 'bile acids') was obtained. After recrystallization from ethyl acetate and drying at about 90° this had m.p. 192–194°, not depressed by authentic cholic acid.

Lactone from Constrictor constrictor. In spite of care taken to avoid conditions leading to lactonization in the isolation of methyl pythocholate from bile, this reaction could not be entirely prevented (e.g. see above, p. 69). In the case of the above species, almost the whole of the crystal crop, from ether, was composed of the lactone, m.p. 262–266°. The yield given in Table 1 is based on the weight of these crystals.

Derivatives of pythocholic acid

Methyl dehydropythocholate. This substance proved unexpectedly difficult to prepare. It was found after a number of experiments that best results were obtained when oxidation was done at about 25°. Methyl pythocholate (0·1 g.) was dissolved by warming in acetic acid (1 ml.). The solution cooled to 25°, was treated gradually with shaking, with 20% CrO_3 (0·3 ml.). After 10 min. at 25°, the solution was diluted and treated with NaCl (excess). After 24 hr. the product, which had crystallized, was collected and recrystallized from dilute ethanol from which it formed white leaflets (50 mg.), m.p. 125–132°. Elution with benzene of a benzene solution of this material from Al_2O_3 (0·5 g.) followed by recrystallization from dilute ethanol gave flat glistening laths (30 mg.) of *methyl dehydropythocholate* (II), m.p. 142–143°; $[\alpha]_D^{20°}$ – 23°±2° in $CHCl_3$ (*c*, 0·55). $[M]_D = -96°$. (Found: C, 72·2; H, 8·8; $C_{25}H_{36}O_5$ requires C, 72·1; H, 8·7%.)

Cholanic acid. The above substance (II) (33 mg., crystalline, partially purified) was added to a solution, in a metal bomb, of Na (40 mg.) in ethanol (2 ml.), with hydrazine hydrate (0·1 ml.). The bomb was sealed and heated at 195–205° for 4 hr. The diluted contents were then acidified with H_2SO_4 and the solid product collected, washed and crystallized three times from dilute ethanol: it (5 mg.) then had m.p. 160–161°, not depressed by authentic cholanic acid prepared by the same process from ethyl dehydrocholate. In another experiment, 20 mg. of methyl dehydropythocholate, m.p. 129–131°, gave 9 mg. of cholanic acid, m.p. 158–159°. The mixed m.p. with an authentic sample (m.p. 161–164°) was 161–163°. (Found: C, 80·1; H, 11·4. Calc. for $C_{24}H_{40}O_2$: C, 80·0; H, 11·1%.)

Dehydropythocholic lactone (V). A solution of pythocholic lactone (0·8 g., m.p. 264–266°) in acetic acid (8 ml.) was cooled in water at room temperature and treated gradually with 20% CrO_3 (1·6 ml.). After 15 min., during which the resulting precipitate was slowly dissolved with occasional shaking, the solution was diluted with about 5 vol. of water. After 24 hr. the solid was collected, washed and dried. It (0·734 g.) was crystallized from dilute ethanol and from l.p./benzene, forming long white needles of *dehydropythocholic lactone*, m.p. 239–241° (decomp.); $[\alpha]_D^{22°}$ +116°±2° in $CHCl_3$ (*c*, 1·0). $[M]_D = +448°$. (Found: C, 74·6; H, 8·6. $C_{24}H_{34}O_4$ requires C, 74·6; H, 8·8%.) Pythocholic lactone had $[\alpha]_D^{22°}$ +52°±1° in $CHCl_3$ (*c*, 1·2). Hence $[M]_D = +203°$.

A sample of deoxycholic acid-ether complex, prepared from cholic acid, had $[\alpha]_D^{22°}$ +39°±1° in approx. 15% (v/v) ethanol/$CHCl_3$ (*c*, 1·15). Hence $[M]_D = +153°$. A highly purified specimen of dehydrodeoxycholic acid had $[\alpha]_D^{22°}$ + 90°±1° in $CHCl_3$ (*c*, 1·3). Hence $[M]_D = +349°$. (Deoxycholic acid had $[\alpha]_D^{22°}$ +48° in pure ethanol.)

Diacetylpythocholic lactone. Pythocholic lactone (0·2 g.) in pyridine (1 ml.) with acetic anhydride (1 ml.) was heated at about 95° for 6 hr. The cooled mixture was diluted with aqueous HCl and the solid precipitate was collected, washed and crystallized from dilute ethanol and l.p./benzene. It was then dissolved in benzene and the solution poured on to Al_2O_3 (1 g.) in a column. The product was eluted with benzene (70 ml.) and recrystallized from l.p./benzene and benzene as long white needles. This *diacetyl pythocholic lactone* had m.p. 197–198°. (Found: C, 70·8; H, 9·0. $C_{28}H_{42}O_6$ requires C, 70·9; H, 8·9%.) This substance was recovered unchanged (m.p. 197–198°, not depressed by the starting material) after treatment with cold 20% CrO_3 in acetic acid.

Monoacetyl pythocholic lactone and derivatives. The lactone (0·12 g.) in pyridine (1 ml.) with acetic anhydride (1 ml.) was kept at about 20° for 16 hr. After dilution with aqueous HCl, the solid was collected, washed and crystallized from l.p./benzene, dilute ethanol and finally benzene; from this it gave white needles. This *monoacetyl pythocholic lactone* had m.p. 243–244°. (Found: C, 72·6; H, 9·3. $C_{26}H_{40}O_5$ requires C, 72·2; H, 9·3%.)

The above substance (27 mg., m.p. 241–243°) in acetic acid (2 ml.) was treated with 20% CrO_3 (0·1 ml.) and the mixture was allowed to stand for 5 min. The solid which separated on dilution was collected, washed and crystallized from dilute ethanol, from which it formed white leaflets. This *ketone*, had m.p. 235–237°, depressed by the original substance. (Found: C, 71·7; H, 8·6. $C_{26}H_{38}O_5$ requires C, 72·5; H, 8·8%.) In spite of the low C analysis, further crystallization did not change the melting point. The *semicarbazone*, prepared in the usual way, had m.p. 259°, with effervescence. (Found, by micro-Kjeldahl and Nesslerization: N, 8·2. $C_{27}H_{41}O_5N_3$ requires N, 8·6%.)

Opening of lactone ring. Dehydropythocholic lactone (0·4 g.) was heated at about 90° for 5 hr. with NaOH (0·1 N; 40 ml.). The compound gradually dissolved to give a yellow solution. After 24 hr. at room temperature this was acidified with H_2SO_4 and the precipitated solid collected, washed and dissolved at once in ethanol. The solution was saturated with diazomethane (in N_2) and left for 16 hr. Evaporation of the ethanol left a colourless gum which slowly crystallized. The crystals were collected and washed with l.p.; they (0·37 g.) had m.p. 123–131°. After two recrystallizations from ethanol, this compound (isomers of VI) had m.p. 156–160°; $[\alpha]_D^{21°}$ +82°±2° in $CHCl_3$ (*c*, 0·83). (Found: C, 72·1; H, 9·2. $C_{25}H_{38}O_5$ requires C, 71·8; H, 9·1%.) The m.p. of the material recovered from the rotation determination was 163–165°.

In spite of the fact that the $[M]_D$ (+343°) of this substance agreed with what would be expected from VI, a number of attempts to convert the compound to methyl dehydropythocholate (II) entirely failed. Likewise, CrO_3 oxidation of the residue left on evaporation of the liquors from the purification of the substance yielded, even after fractionation on an Al_2O_3 column, only about 4 mg. of crystalline material of m.p. 109–111°. In another similar experiment beginning with the dehydrolactone it did prove possible, however, to isolate a very small yield of methyl dehydropythocholate (20 mg., m.p. 129–131°) which was converted to cholanic acid (see above). Other experiments suggested that it might be possible to open the lactone ring by milder alkali treatment, with ethanol as a solvent.

Partial oxidation, followed by reduction, of methyl pythocholate (cf. Haslewood, 1943). This ester (0·2 g.) was dissolved by warming with acetic acid (2 ml.) and sodium acetate ($CH_3COONa.3H_2O$; 0·4 g.). A solution of K_2CrO_4 (31·7 g./100 ml.; 0·4 ml.) was added slowly with shaking, followed by acetic acid (4 ml.). The mixture was shaken at intervals during 10 min., until all the precipitate had dissolved. It was then left with occasional shaking at about 23° for 24 hr., after which it was diluted with water and treated with NaCl (excess). After 24 hr. the organic product was extracted with ether and this was washed with water, dilute ammonia, water and dried (Na_2SO_4). The gummy residue left on evaporation of the ether was transferred to a small metal bomb and dissolved in ethanol (8 ml.) with sodium (0·16 g.) and hydrazine hydrate (0·4 ml.). The bomb was sealed and heated at 200–204° for 4·5 hr. The contents of the cooled bomb were then diluted with water and acidified with H_2SO_4. The precipitated solid was collected, washed with water and dissolved in ethanol. Evaporation of this left a residue which was dissolved in a little ether and kept at about 5°, when crystals separated. These (18 mg., m.p. approx. 190°) were collected after some weeks, washed with cold ether and recrystallized from l.p./ethanol, from which small colourless regular prisms (8 mg.) separated. This *deoxypythocholic acid* had m.p. approx. 160°, depending on the rate of heating. (Found: C, 73·6; H, 10·3. $C_{24}H_{40}O_4$ requires C, 73·5; H, 10·2%.) The original ethanol liquors on evaporation left a residue which, with dilute acetic acid, gave crystals (4 mg.) which after two recrystallizations from dilute ethanol gave needles of m.p. 155–159°, not depressed by cholanic acid.

DISCUSSION

Biological. The present results lend considerable support to the view that pythocholic acid will be found to be characteristic of the Boidae, for it has now been found in four different genera of the family. Cholic acid, which forms the chief bile acid in a number of snakes, is also to be found in some Boidae. In *Boa canina*, only about 30% of the 'bile acids' was accounted for, and this suggests that some as yet unidentified bile acid may be present. Snake bile doubtless contains a variable amount of neutral and acidic lipid which may be regarded as in the course of excretion via the liver, and such material will be included in the weight of the 'bile acids'. However, it would be surprising if it constituted as much as 70% of this weight; especially as in two other species, only 30% of the 'bile acids' was not accounted for as pythocholic acid.

It is not of course possible to say whether pythocholic acid should be regarded as a unique bile acid, formed perhaps as the result of a genetic mutation in snakes producing (say) cholic acid, or whether the Boidae have retained pythocholic acid as a legacy from the limbed reptiles from which they may be presumed to have evolved. In considering this question, however, one may notice that the only other lactone-forming bile acids so far said to exist have been isolated from the chelonian reptiles *Amyda japonica* (Yamasaki & Yuuki, 1936) and *Emys orbicularis* (Kim, 1939).

It will be of great interest to compare the chemical constitution, when it is known, of these lactones with that of pythocholic lactone.

If pythocholic acid, is, so to speak, an evolutionary legacy, then perhaps it is being replaced in the Boidae by the generally occurring cholic acid.

Chemical. If the, so far as is known, universal occurrence in the bile acids of a 3α-hydroxyl group is accepted in the present case it may be said with some confidence that pythocholic acid is probably 3α:12:16 (or 15)-trihydroxycholanic acid. Molecular models show that lactone formation is hardly possible with any secondary hydroxyl group other than one at C_{12}, C_{15} or C_{16}. Hence if the evidence for OH groups at C_3 and C_{12} in the lactone is considered strong, the remaining hydroxyl group in pythocholic acid can only be at C_{15} or C_{16}. The chemical and optical evidence, whilst perhaps slightly favouring C_{16}, does not enable us to choose with confidence between these positions, and hence we tentatively formulate pythocholic acid as (I) and its lactone as (IV).

SUMMARY

1. Approximate figures are given for the amounts of cholic acid and pythocholic acid in the bile of seven species of Boidae. The possible significance of the occurrence of these bile acids in boa and python bile is discussed.

2. Pythocholic acid is shown to be a trihydroxycholanic acid. The three hydroxyl groups are secondary and are considered to be probably at C_3, C_{12} and C_{15} or C_{16} in the steroid nucleus. Various derivatives of pythocholic acid and its lactone have been prepared.

We offer our gratitude and thanks to Dr W. C. Osman Hill, Dr R. E. Rewell, Mr W. E. Lawrence and the Zoological Society of London, without whose help in supplying gall-bladders this work could not have been undertaken.

We also acknowledge a grant from the Medical Research Council to one of us (V. M. W.).

REFERENCES

Barton, D. H. R. & Klyne, W. (1948). *Chem. & Ind.* p. 755.
Charonnat, R. & Gauthier, B. (1947). *C.R. Acad. Sci., Paris*, **224**, 279.
Haslewood, G. A. D. (1943). *Biochem. J.* **37**, 109.
Haslewood, G. A. D. & Wootton, V. (1950*a*). *Biochem. J.* **46**, x.
Haslewood, G. A. D. & Wootton, V. (1950*b*). *Biochem. J.* **47**, 584.
Kim, C. H. (1939). *J. Biochem., Tokyo*, **30**, 247.
Shoppee, C. W. (1949). *J. chem. Soc.* p. 1671.
Smith, M. (1927). *List of Vertebrated Animals exhibited by the Zoological Society of London.* Vol. 2. *Amphibia and Reptiles.* London: Zoological Society.
Yamasaki, K. & Yuuki, M. (1936). *Hoppe-Seyl. Z.* **244**, 173.