SUMMARY

1. Purified C-esterase (from hog's kidney) is stable within the range pH 5·6–10·2. After exposure of the enzyme to these extremes of pH, its activity can be fully recovered by readjustment of the pH to 8·0.

2. The activating effect of organic mercurials is pH-dependent. The changes are immediate and completely reversible.

3. Some of the preparations of C-esterase appear to contain a dialysable cofactor, which is lost rapidly by exposure of the preparations to pH 5·6 or to pH 10·2.

4. The pH–activity curves for phenyl acetate and its *p*-methoxy derivative exhibit an optimum around pH 8. In this respect, C-esterase behaves similarly to esterases of the A- and B-type.

5. In contrast, the rate of the enzymic hydrolysis of *p*-nitrophenyl acetate increases steadily up to the experimental limit of pH 9·5.

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Condensed Tannins

2. BIOGENESIS OF CONDENSED TANNINS BASED ON LEUCO-ANTHOCYANINS*

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The previous work by Roux (1957*a*, *b*, 1958*a*) and by Roux & Evelyn (1958) showed the association of monomeric and complex leuco-anthocyanins of similar structural pattern in those typical 'condensed tannins' present in extractives of black-wattle bark and quebracho heartwood. Chromatographic evidence illustrated the apparent conversion of monomeric into the complex polymeric leuco-anthocyanins. The present study provides details of the chromatographic identification, isolation and characterization of some of these new monomeric leuco-anthocyanins. By means of a study of molecular weight evidence is also provided to support the concept of their conversion into complex tannins during radial translocation within the plant.

EXPERIMENTAL AND RESULTS

All melting points are uncorrected. Analyses of C, H, methoxyl and acetyl are by Weiler and Strauss, Oxford. Tannin analyses by the hide-powder (shake) method are by Miss S. Whitelaw of these Laboratories.

* Part 1. Roux & Evelyn (1958).

Variations in quebracho woods. The wood of *Schinopsis balansae* from Vera, Santa Fe, and also from unknown areas in the Argentine, appeared uniformly dark-reddish brown in cross-section, without differentiation into heartwood and sapwood areas. A single specimen obtained recently from the same source was clearly divided into a white sapwood and a light-amber heartwood.

A cross-section of *Schinopsis quebracho-colorado* [syn. *S. lorentzii*; see Barkley & Meyer (1950) for discussion on nomenclature] collected from Atamisqui, Santiago del Estere, (specimen A) showed sharp subdivision into a yellow sapwood and a deep-amber, almost black, central-heartwood region (Roux, 1958*a*). Other samples obtained from the same source but from unknown regions of the Argentine, and also from the Union Department of Forestry plantations at Hanglip, Northern Transvaal (specimens B), differed in that a white sapwood 2 in. wide surrounded the light amber–brown central heartwood. These are almost identical in appearance with the divergent sample of *S. balansae*.

Examination of extractives of Schinopsis *spp.*

Isolation of a new leuco-fisetinidin. Dry wood (100 g.) of *S. quebracho-colorado* (specimen A, a sapling of 7–8 cm. diam.) was cut into chips and ground into a fine powder with a Wiley mill. The powder was extracted first with

1·05 l. of methanol (three extractions with 350 ml. each for 24 hr.), and finally exhaustively with 1·75 l. of acetone–water (1:1) (five extractions for 48 hr. each). The extracts were streaked on to paper-chromatographic sheets (Whatman no. 3) and developed with 2% acetic acid. The monomeric leuco-anthocyanin migrated in advance of the polymeric units (Roux & Evelyn, 1958), and could be located by spraying a strip cut from the edge of the sheet with the toluene-*p*-sulphonic acid reagent. Subsequently it was located merely by reference to certain fluorescent bands on paper sheets. The bands were cut out and eluted with 70% ethanol. From a concentrate of the eluents the leuco-anthocyanin crystallized easily in long thin needles (2 g.). Simultaneous elution of the tannins with lower R_F values showed that the leuco-anthocyanin formed 10% of the tannins present, and the yield was also 2% on wood weight.

The compound, which is sparingly soluble in cold water, was recrystallized from water as a white dihydrate (Found: C, 55·8; H, 5·6; loss at 110°, 11·0. Calc. for $C_{15}H_{14}O_6,2H_2O$: C, 55·2; H, 5·6; H_2O, 10·6%), m.p. 130° with reddening after softening at 110°. A green colour with $FeCl_3$ indicated the presence of a catechol nucleus. On paper chromatograms its R_F in 2% acetic acid (0·50) and scarlet colour developed with toluene-*p*-sulphonic acid reagent suggested the presence of a flavan-3:4-diol structure (Roux, 1958*b*; Roux & Evelyn, 1958). In a partitioning mixture, e.g. butan-1-ol–acetic acid–water (4:1:5), it migrated at the same rate (R_F 0·76) as (+)-catechin, indicating the probable presence of the same number of hydroxyl groups on the C_{15} skeleton.

The compound was easily converted into the anthocyanidin, fisetinidin, in the presence of 3N-HCl in propan-2-ol solution under pressure at 100°. The anthocyanidin was identified by the methods previously outlined (Roux, 1957*a*, *b*; Roux & Evelyn, 1958). The comparative yields of fisetinidin formed from similar leuco-anthocyanins are represented in Table 1. Fusion with KOH under controlled conditions (Roux, 1952) resulted in degradation into β-resorcylic and protocatechuic acids.

The leuco-fisetinidin from quebracho wood shows the same absorption maximum (282 mμ) as the 7:3′:4′-trihydroxyflavan-3:4-diol obtained by the catalytic (platinum oxide) hydrogenation of fustin (Freudenberg & Roux, 1954; Roux & Freudenberg, 1958), and also a similar $\epsilon_{max.}$ (6521) to this synthetic flavan-3:4-diol (6414). Its specific rotation in methanol, $[\alpha]_D^{25} - 11{\cdot}1 \pm 1{\cdot}1°$ (*c*, 1·08) distinguished it from (+)-mollisacacidin (Keppler, 1957) and also from the hydrogenation product of fustin $[\alpha]_D^{23} - 2{\cdot}4°$ (Roux & Freudenberg, 1958). Anhydrous (−)-leuco-fisetinidin readily reabsorbs moisture from the atmosphere, and drastic drying under vacuum at 110° over P_2O_5 is required for the removal of water of crystallization. The compound assumes a pink colour under these conditions (Found: C, 61·6; H, 5·0. Calc. for $C_{15}O_{14}O_6$: C, 62·1; H, 4·9%).

7:3′:4′-*Trimethoxyflavan*-3:4-*diol*. (−)-Leuco-fisetinidin (500 mg.) in 50 ml. of methanol at −5° was methylated with 45 ml. of ethereal diazomethane generated from 10 g. of nitrosomethylurea. The mixture was kept at −5° overnight, and reduced to small volumes by evaporation and poured into water. The white product (0·4 g.) failed to crystallize from conventional media such as absolute ethanol or methanol, and settled out as a sludge on cooling. The compound crystallized as a hydrate in fine needles (m.p. 81–86°) from 50% aq. ethanol. After drying overnight over silica gel the hydrate contained 1·5 mol. prop. of water (Found: C, 60·0; H, 6·6; OMe, 25·9. Calc. for $C_{18}H_{20}O_6,1{\cdot}5H_2O$: C, 60·2; H, 6·3: OMe, 25·9%). One mol. prop. of water was easily lost by drying for 2 hr. at 60° (Found: C, 63·2; H, 6·2. Calc. for $C_{18}H_{20}O_6,0{\cdot}5H_2O$: C, 63·3; H, 6·2%). The remaining water was strongly held, and drying for 6 hr. at 70° over P_2O_5 was required to give the anhydrous form. (Found: C, 65·1; H, 6·2; OMe, 28·0. Calc. for $C_{18}H_{20}O_6$: C, 65·1; H, 6·1; OMe, 28·0%). The anhydrous trimethylether settles slightly at 81–84°, sharply at 129° and the melt liquefies completely at 134°. Under the conditions of Pigman, Anderson, Fischer, Buchanan & Browning (1953), and in the presence of HCl, the trimethoxyflavan-3:4-diol is converted into a scarlet pigment, presumably 3-hydroxy-7:3′:4′-trimethoxypyrilium chloride, and the corresponding flavonol (paper chromatogram).

3:4-*Diacetoxy*-7:3′:4′-*trimethoxyflavan*. The trimethylether (500 mg.) was dissolved with warming in 5 ml. of acetic anhydride, and 1 g. of freshly dried sodium acetate added. The mixture was slowly brought to the boil, and kept near the boil for 5–10 min. It was then cooled, and poured into 50 ml. of iced water and left overnight. The dry solid (0·45 g.) was repeatedly recrystallized from ethanol. The product had m.p. 102–104° (Found: OMe, 22·8; $CO.CH_3$, 20·9. Calc. for $C_{22}H_{24}O_8$: OMe, 22·4; $CO.CH_3$, 20·7%).

3:4:7:3′:4′-*Penta-acetylflavan*. (−)-Leuco-fisetinidin dissolved in 5 ml. of pyridine was treated with 5 ml. of acetic anhydride. Reaction occurred spontaneously at room temperature, and after 2–3 hr. the mixture was poured into iced water. A pure white granular product (0·53 g.) separated, which failed to crystallize from ethanol, methanol or other solvents (Found: C, 59·7; H, 5·1; $CO.CH_3$, 41·9. Calc. for $C_{25}H_{24}O_{11}$: C, 60·0; H, 4·8; $CO.CH_3$, 43·0%).

Table 1. *Yields of fisetinidin from monomeric leuco-fisetinidins*

Source	Weight of leuco-anthocyanin (mg.)	Yield of fisetinidin chloride (%)
Schinopsis quebracho-colorado	3·5	15·7
	2·4	17·3
Acacia mollissima	3·2	20·0
	4·7	13·2
Reduction product of fustin	6·9	14·5
	4·8	13·6

The extractives of woods of *Schinopsis* spp. were initially examined for leuco-anthocyanins by two-way paper chromatography and the toluene-*p*-sulphonic acid-spraying reagent (Roux, 1957*b*). Chromatograms of *S. quebracho-colorado* showed the presence of a monomeric leuco-fisetinidin in high concentration in the sapwood of specimen A, and in the light-amber heartwoods of specimens B. Complex leuco-fisetinidins were present in all specimens. The wood of the uniformly dark-coloured samples of *S. balansae* contained complex leuco-fisetinidins of similar distribution to those

present in *S. quebracho-colorado*. The divergent sample of *S. balansae* also contained monomeric leuco-fisetinidin at the sapwood–heartwood junction, as also recorded by King & White (1957*a*, *b*) for both species of *Schinopsis*.

In the adult trees, of about 35 cm. diameter, of *S. quebracho-colorado* and *S. balansae* which contain outer sapwood 4–5 cm. wide, the leuco-fisetinidin is present in the sapwood only in low concentration, and in the outer margin of the heartwood, 1 cm. wide, in high concentration. The central heartwood of about 22 cm. diameter contains only polymeric condensed tannins, amongst which complex leuco-fisetinidins are present, and overall the percentage of monomeric leuco-fisetinidins is therefore much lower than in the sapling examined.

The outer sapwood (Mezey, 1947; Howes, 1953) and also the heartwood edge (in a typical log received by us from the Argentine) is removed from the heartwood core before commercial extraction. This is probably one explanation of the complete absence of monomeric leuco-fisetinidin in the commercial extract (see Freudenberg & Maitland, 1934; Roux, 1958*b*). The uniformly dark-coloured specimens of *S. balansae*, showing no sapwood, contained typical polymeric but no monomeric forms (Roux, 1958*a*) and may represent varietal forms of the same species.

Radial sampling of S. quebracho-colorado *heartwood.* The heartwood (specimens B) was sampled by drilling (Fig. 1), the concentration of polyphenolics being very low in the sapwood. The wood of these specimens also showed the radial increase in complexity previously found in specimen A (Roux, 1958*a*), although emphasis on the polyphenolic tannins of low R_F (zero R_F in 2 % acetic acid) in the central heartwood was not quite as pronounced as in specimen A. The percentages of extractives and 'non-tannins' of the radial samples, the yield of fisetinidin from each and the average molecular weight of the tannins (Evelyn, 1954*a*, *b*; Roux & Evelyn, 1958) were estimated (Table 2).

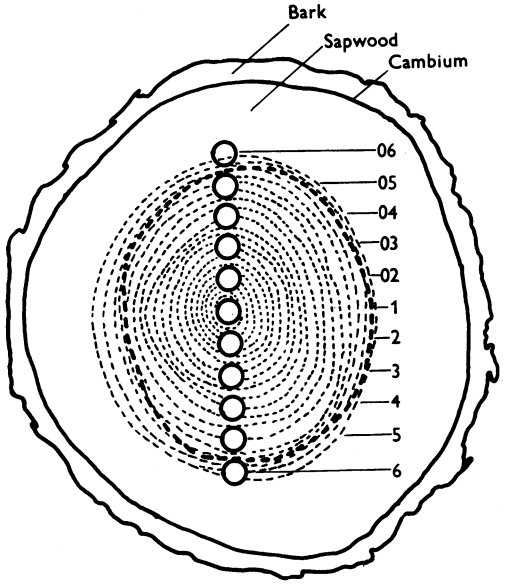

Fig. 1. Sampling positions on the cross-section of the wood of *Schinopsis quebracho-colorado*. Samples were obtained by drilling. Drawn to scale, to illustrate the annual rings (light circular broken lines) within the 'heartwood' area (heavy circular broken line).

Table 2. *Non-tannin content, yields of anthocyanidins and average molecular weights of tannins present in radial samples from the wood of* Schinopsis quebracho-colorado

See Fig. 1 for sampling position. Percentage of extractives is based on dry wt. of wood samples. Wood extracts 06 and 6 contain mainly sugars and simple phenolic constituents. The percentage of 'tannins' in the extractives may be obtained by subtraction of the value for 'non-tannins' from 100 %. Variable results for conversion into anthocyanidins are due to a variety of known and unknown factors influencing the generation reaction (Pigman *et al.* 1953). For central-heartwood samples 04–4, where monomeric leuco-anthocyanins are largely absent, the conversion based on 'tannin' content varies within limits 4·2–8·2 %. Calculations are based on yield of fisetinidin from anhydrous extractives (see Roux & Evelyn, 1958).

Sampling position	Extractives (%)	Non-tannins (% of extractives)	Conversion into anthocyanidins (%)	Average mol.wt. of tannins
06	3·6	—	0·5	—
05	21·3	25·0	4·2	655
04	22·3	17·2	5·5	726
03	24·4	15·1	4·4	801
02	25·3	10·6	3·8	953
1	20·0	9·9	4·5	1039
2	22·1	11·8	6·3	919
3	22·0	15·1	5·5	846
4	20·4	20·6	6·5	751
5	9·8	39·0	4·6	659
6	3·2	—	1·4	—

Leuco-anthocyanins of the bark of Schinopsis quebracho-colorado. The methanol extractives of the dried bark gave an intense scarlet coloration with HCl, under the conditions of Pigman *et al.* (1953), which was due to delphinidin, and from two-way paper chromatograms of the extracts complex leuco-delphinidins of low R_F appeared to be present in addition to mobile non-leuco-anthocyanin constituents of higher R_F.

Examination of extractives of Acacia *spp.*

Monomeric leuco-anthocyanins of black-wattle-bark extractives. Freshly stripped black-wattle (*Acacia mollissima*) bark was sliced into thin slivers with a stainless-steel knife, and extracted at 0–5° with methanol. The methanolic extract was streaked on to sheets of Whatman no. 3 paper, and the chromatogram was developed with 2% acetic acid. Strips cut from each sheet, corresponding to R_F 0·50–0·60, were eluted with 70% ethanol. The concentrated eluent was examined by two-dimensional chromatography with butan-2-ol saturated with water and then with 2% acetic acid. Of the reducing areas present in this fraction only two, LF (R_F 0·68 and 0·50 in the solvents respectively) and LR (R_F 0·54 in each solvent) gave the scarlet leuco-anthocyanin reaction with the toluene-*p*-sulphonic acid reagent (Roux, 1957*b*). The $FeCl_3$ spray gave a green coloration with LF (catechol unit), whereas LR afforded a blue coloration (pyrogallol unit). The position of LF corresponded exactly with leuco-fisetinidin or 7:3′:4′-trihydroxyflavan-3:4-diol, and LR with leuco-robinetinidin or 7:3′:4′:5′-tetrahydroxyflavan 3:4-diol on paper chromatograms, when artificial mixtures of these reference compounds (Freudenberg & Roux, 1954; Roux & Freudenberg, 1958) were made with this wattle fraction.

The wattle fraction obtained above (R_F 0·5–0·6 in 2% acetic acid) was streaked on Whatman no. 3 paper, and the chromatogram was developed with butan-1-ol–acetic acid–water (12:3:5, by vol.). After development, fractions corresponding with LF and LR were cut. From the LF fraction fisetinidin and from the LR fraction robinetinidin were generated by treatment with HCl (Pigman *et al.* 1953) and identified as before. The monomeric leuco-fisetinidin and leuco-robinetinidin constitute some of the minor constituents of wattle extract, and their concentration is considered too low to permit isolation in sufficient quantity for detailed study.

Leuco-anthocyanins of the bark of Acacia melanoxylon. The methanolic extractives of the fresh bark gave the same results as those given by the dried bark of *S. quebracho-colorado* (see above).

DISCUSSION

The (−)-7:3′:4′-trihydroxyflavan-3:4-diol from the sap- and heart-woods of *Schinopsis quebracho-colorado* differs in its melting-point and the melting-points of its derivatives from the (+)-leuco-fisetinidin (mollisacacidin) obtained by Keppler (1957) from the heartwood of *Acacia mollissima*, and also from the hydrogenation product of dihydrofisetin (Roux & Freudenberg, 1958). These leuco-fisetinidins, on the other hand, show similar behaviour in the way in which they redden at and above 110°, and in the non-crystalline nature of their penta-acetyl derivatives. The (−)-leuco-fisetinidin from quebracho wood was also characterized by Freudenberg & Weinges (personal communication from Professor K. Freudenberg), and was noticed by King & White (1957*a*, *b*), although their tentative identification of this constituent as an amorphous monoglucoside is not exact. The trimethyl derivative, which requires the presence of moisture in order to promote crystallization, differs markedly in this respect from the similar derivatives of other leuco-fisetinidins.

The rapid genesis of the monomeric leuco-fisetinidin (also recorded by King & White, 1957*a*, *b*) in the sapwood edge (specimen A), or in the sapwood and sapwood–heartwood junction (specimens B) of *S. quebracho-colorado* and in the sapwood–heartwood area of one specimen of *S. balansae*, and its rapid and progressive transformation into condensed tannins of the leuco-fisetinidin type in the central-heartwood area, provides presumptive evidence of the biogenesis of complex tannins from monomeric leuco-anthocyanins (Roux, 1957*a*, *b*; 1958*a*), and strongly suggests that the long-sought precursor of the quebracho tannins is, in fact, leuco-fisetinidin (Roux, 1958*b*). A study of the radial samples of *S. quebracho-colorado* by molecular-weight methods (Table 2) shows that the transition of monomeric leuco-fisetinidin into progressively more complex leuco-fisetinidins from the outer to the central heartwood is accompanied (*a*) by a gradational increase in molecular weight from 650, for the outer heartwood, to 1050, for the central heartwood, and (*b*) by a progressive reduction in the non-tannins present, or by an increase in the tannin:non-tannin ratio, but with no definite trend in the total percentage of extractives present in each sample. The percentage of anthocyanidins generated from quebracho tannins (Table 2) compared with the yield from monomeric leuco-fisetinidins (Table 1) indicates a total leuco-anthocyanin content of 20–35% in *S. quebracho-colorado*. The conversion of monomeric into polymeric leuco-anthocyanins is therefore one of the important transformations which occurs in what is obviously a complex mixture of tannin constituents

(see King & White, 1957*a*, *b*). The high percentage (10%) of monomeric leuco-fisetinidin present in the outer heartwood- or sapwood-tannin mixture, and the relative completeness of its transformation into complex leuco-anthocyanin tannins in the central heartwood (Roux, 1958*a*), also supports this view. Carbohydrate non-tannins and also polyphenolic half-tannins (see Roux, 1957*c*) are apparently converted into tannins during this process (Table 2).

In most barks, e.g. black-wattle bark (*A. mollissima*), a greater degree of differentiation and complexity in cell structure exists, and attempted layerwise radial sampling from the cambial to cortical layers did not show the transformations evident in some heartwoods. It is, nevertheless, interesting to record the association of monomeric and complex leuco-anthocyanins of the robinetinidin and fisetinidin types in the whole fresh-bark extract of *A. mollissima*.

Although heart-wood formation is a complex phenomenon, and heartwoods are somewhat different anatomically in the angio- and gymnosperms, Erdtman (1952, 1955, 1956) has found that amongst the conifers, heartwood formation is controlled from the cambium. Heartwood formation ceases, for example, on injury of the cambium and is re-established once the cambial layer has healed. Furthermore, heartwood and bark constituents appear to originate in the cambium and are distributed both centripetally (via medullary rays) and centrifugally in many instances. Hillis (1956*a*, *b*) described identical concepts for various *Eucalyptus* species, where the leuco-anthocyanins of the xylem are considered to originate in the leaves, and to represent intermediate precursors of heartwood and bark extractives. King & White (1957*a*, *b*) concluded that the 'hydrolysable' tannins of the leaves of *Schinopsis* spp. constitute the phenolic raw material, which is ultimately converted into typical 'condensed' tannins of the heartwood. Catechin and leuco-anthocyanins participate in this conversion. They, however, overlooked (White, 1956, 1957; King & White, 1956) the leuco-anthocyanidin nature of some of these condensed tannins (see Roux, 1957*a*, *b*; 1958*a*).

Considering only differences in the leuco-anthocyanins present in the barks and heartwoods of *Schinopsis* spp. (leuco-delphinidins and mainly leuco-fisetinidins, Roux, 1957*a*), of *Acacia melanoxylon* (leuco-delphinidins and melacacidins, King & Bottomley, 1954) and of *A. mollissima* and closely related species (leuco-robinetinidins + leuco-fisetinidins and mollisacacidin, Keppler, 1957), it appears that precursor material originating from the cambium is subjected to the action of two different and highly specific enzymic systems in the heartwood and in the bark, after centripetal and centrifugal translocation. These enzymes vary in activity, e.g. monomeric melacacidins and mollisacacidins persist into the central heartwoods of *A. melanoxylon* and *A. mollissima* respectively, whereas in heartwoods of *Schinopsis* rapid conversion of the monomeric leuco-fisetinidin occurs, and also the polymeric forms appear to undergo conversion (Table 2 and Roux, 1958*a*). This rapid enzymic conversion persists throughout the heartwood of *S. quebracho-colorado*, showing that in this instance even the central-heartwood cells still contain active-enzyme systems. Differences between the high average molecular weights (1460: ranging from 700 to 2300, Roux & Evelyn, 1958) of commercial quebracho extract from adult (100–175 years) trees, and the much lower average (650–1050) from the young tree (42 years) examined, suggests that progressive molecular-weight increases in the central heartwood occurs even during very advanced stages of growth in *Schinopsis* spp. The shape of the heartwood in *S. quebracho-colorado* usually follows the shape of the cambium rather than that of the annual rings. The concept of the cambium as common origin of heartwood and bark tannins finds support in the observations that whereas the cambium of *A. mollissima* contains no tannins (Sidey, 1953), both the oldest and youngest strippable barks of *A. mollissima* contain complex tannins of almost equally high average molecular weights (Evelyn, 1956).

The above instances of the association of monomeric ('non- or half-tannins') and complex leuco-anthocyanins ('condensed tannins') in commercially important vegetable extracts used for tannage, must be linked directly with the high correlation between the occurrence of leuco-anthocyanins and 'botanical' tannins in nature, and with their exceptionally wide distribution (Bate-Smith & Lerner, 1954; Bate-Smith, 1957).

SUMMARY

1. Details are given of the isolation and characterization of a monomeric (−)-leuco-fisetinidin from the wood extractives of *Schinopsis quebracho-colorado*, and of the identification by chromatography of a monomeric leuco-robinetinidin and a leuco-fisetinidin in the fresh-bark extract of *Acacia mollissima* ('wattle' extract).

2. Differences in bark and heartwood leuco-anthocyanins in these and other species suggest that the cambium furnishes the common precursors of both bark and heartwood tannins, and that different and very specific enzymic systems effect conversion into condensed tannins in each portion of the plant.

3. Evidence for the biosynthesis of condensed tannins from monomeric leuco-anthocyanins is presented.

4. Conversions persist into the central heartwood, suggesting that these cells still contain active-enzyme systems at an advanced age, even after heavy deposition of tannin.

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Studies in the Bile Acids

3. THE CONJUGATED BILE SALTS OF CERTAIN PRIMATES*

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In a previous paper, the non-ketonic acids of human bile were described (Wootton & Wiggins, 1953). The major constituent was shown to be chenodeoxycholic acid (3α:7α-dihydroxycholanic acid) and it was suggested that the presence of a high proportion of this acid might be characteristic of the human species. To test this suggestion, a series of primate biles have now been examined.

The human-bile analyses reported in 1953 were done by hydrolysing the conjugated bile salts and submitting the bile acids produced (after methylation) to chromatographic separation and identification by infrared spectroscopy. No distinction was therefore made between glycine-conjugated and taurine-conjugated acids. However, Ahrens & Craig (1952) have developed a countercurrent method capable of separating these two classes of conjugates and this procedure has been applied as the initial stage of the primate-bile analyses. The separated fractions of the bile have then been carried separately through the remainder of the analysis. This complete method has been applied to samples of gall-bladder bile derived from the human (three specimens), the chimpanzee (*Pan satyrus*), the white-nosed monkey (two specimens of *Cercopithecus nictitans mortinii*), the mona monkey (*Cercopithecus mona*) and the tantalus guernon (*Cercopithecus aethiops tantalus*). The specimens of monkey bile were kindly provided by Professor G. A. D. Haslewood.

* Part 2: Wootton & Wiggins (1953).