388. *The Ether-soluble Constituents of Sarsaparilla Root. Part II.*

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The liquid fraction of the non-saponifiable matter obtained from the ether-soluble material of sarsaparilla root consists of a highly complex mixture of unsaturated alcohols and hydrocarbons, probably containing azulene. Treatment of two of the more volatile fractions with 3 : 5-dinitrobenzoyl chloride gives traces of a *substance*, $C_{15}H_{11}O_6N_2$, m. p. 111°. From the alcoholic components of the mixture, a hexenol and an octadecenol have been isolated as their crystalline *pyruvic ester semicarbazones*.

In continuation of our examination of the ether-soluble material of sarsaparilla root we have made a partial inspection of the non-saponifiable residues contained in filtrate A′ and fraction E (see scheme in previous paper; Simpson and Williams, J., 1937, 735) after removal of the mixed sterol benzoates. These residues, after hydrolysis, consisted of a very dark, viscous oil, comprising 8% of the total ether-soluble material. The oil was characterised by a fragrant and extremely persistent odour, which is entirely absent from the original ether-soluble material but immediately becomes noticeable on saponification of any of the fatty fractions.

The more volatile constituents of the oil could be readily distilled without decomposition; these were eventually separated into a number of sub-fractions (Table I).

The odoriferous components of the root were contained almost exclusively in sub-fractions 1—6. In the hope of achieving their identification, representative samples were treated with (i) nitrosyl chloride, (ii) catalytically activated hydrogen (which caused almost complete disappearance of the odour), and (iii) (ii), followed by permanganate oxidation. Although reactions readily occurred in every case, no crystalline product could be isolated. Esterification of sub-fractions 3 and 6 with 3 : 5-dinitrobenzoyl chloride, however, yielded a minute amount of an unidentified *substance*, m. p. 111°, of empirical formula $C_{15}H_{11}O_6N_2$.

The (primary) alcoholic components of sub-fractions 1—6 were then isolated collectively with phthalic anhydride and converted into their pyruvic esters. Treatment of this ester mixture with semicarbazide acetate furnished a crystalline *pyruvic ester semicarbazone*,

$C_{10}H_{17}O_3N_3$, m. p. 115°; the ester is therefore that of a hexenol. A primary hexenol has been isolated in similar fashion by Ruzicka and Schinz (*Helv. Chim. Acta*, 1935, **18**, 381) from the odoriferous components of violet leaf oil; the melting point of the violet leaf ester given by these authors is almost identical with that of our sarsaparilla compound, but their analysis indicates contamination with the ester-semicarbazone of *n*-hexyl alcohol. As the melting point of the latter compound is 144° (Ruzicka and Schinz, *loc. cit.*), the identity of the two hexenols seems doubtful.

From the mixture of alcohols isolated from the higher sub-fractions (15, 17—20) we have obtained a second *pyruvic ester semicarbazone*, $C_{22}H_{41}O_3N_3$, m. p. 137°; the formula of the related alcohol is therefore $C_{18}H_{36}O$. The possibility that this octadecenol might be the biological precursor, or product, of oleic acid seems to be discountenanced by its method of isolation, which, from analogy with the work of Chibnall, Piper, Pollard, Smith, and Williams (*Biochem. J.*, 1931, **25**, 2095) on long-chain saturated alcohols, indicates that it is probably a secondary alcohol. Lack of material has prevented structural work on this compound.

The mixed non-alcoholic components of the higher sub-fractions (15, 17—20) were submitted to selenium dehydrogenation. A vigorous reaction occurred at 290—300°, indicating the probable polyenic or hydroaromatic character of the material, but no solid picrate or trinitrobenzene compound could be obtained from any fraction of the product.

Indications were obtained of the presence of azulene in sub-fraction 16, but no positive identification could be realised.

In Part I we drew attention to certain discrepancies between the results therein reported and the earlier findings of Power and Salway (J., 1914, **105**, 201). The present investigation has emphasised this dissimilarity, for no mention is made by these authors of the material which we have now examined or of any of the products which we have isolated.

EXPERIMENTAL.

(Melting points are uncorrected.)

The filtrates from the sterol benzoates previously isolated were evaporated, and the residual material refluxed for 5½ hours with its own weight of potassium hydroxide in 90% alcohol (8·5 vols.). After removal of two-thirds of the alcohol by distillation the solution was largely diluted with water and extracted with ether. The washed and dried extract was evaporated, and the residue roughly fractionated at 1 mm., 100 g. yielding the following fractions: (I) b. p. — 120°, 17·1 g.; (II) 120—200°, 19 g.; (III) 200—220°, 14·6 g.; (IV) 220—230°, 3·3 g.; residue, approx. 40 g. Redistillation of fraction (I) at 20 mm. gave (Ia) b. p. — 70°, 3·0 g.; (Ib) 70—120°, 4·0 g.; (Ic) 120—130°, 5·7 g.; (Id) 130—140°, 2·2 g. Fractions (Ia) and (Ib) were combined and carefully refractionated, yielding sub-fractions 1—10 (Table I). Fraction (II) (after removal of solid paraffins which separated on standing; m. p. 46—47° after recrystallisation from acetone) was combined with fractions (Ic), (Id), and the residue from sub-fraction 10, refractionation of the mixture giving sub-fractions 11—20 (Table I).

TABLE I.

Sub-fraction.	B. p.	Pressure.	Wt. (g.).	Sub-fraction.	B. p.	Pressure.	Wt. (g.).
1	—75°	20 mm.	0·69	11	131—138°	20 mm.	1·80
2	81—83	,,	0·34	12	138—144	,,	1·61
3	83—86	,,	0·85	13	148—150	,,	1·57
4	90—95	,,	0·36	14	150—157	,,	3·86
5	95—96	,,	0·31	15	140—150	1 mm.	1·92
6	100—105	,,	0·70	16	150—160	,,	1·90
7	105—110	,,	0·44	17	160—170	,,	1·87
8	110—115	,,	0·46	18	170—180	,,	2·30
9	120—122	,,	0·28	19	180—190	,,	2·97
10	130—131	,,	1·20	20	190—200	,,	2·24

Found for (3): C, 77·4; H, 10·4; active H (Zerewitinoff determination), 0·37%. Found for (5): C, 77·5; H, 10·2%. Found for (6): active H, 0·39%. Found for (9): C, 81·3; H, 10·1; active H, 0·23%. Found for (10): active H, 0·16%. The reactions of (3), (6), (9), and (10) with tetranitromethane were positive.

After sub-fractions 1—6, which were almost colourless, the colour rapidly deepened up to sub-fraction 16, which was deep blue; beyond this point the colour faded somewhat.

Substance, m. p. 111°.—A sample of sub-fraction (3) was dissolved in pyridine and heated with its own weight of 3 : 5-dinitrobenzoyl chloride for 3 hours on the steam-bath. The solution was decomposed with ice and dilute sulphuric acid and worked up in the usual manner; the *product* separated from benzene–light petroleum in prismatic needles, m. p. 110—111° (Found : C, 56·7; H, 3·1. $C_{15}H_{11}O_6N_2$ requires C, 57·1; H, 3·4%). This substance was also obtained in very small amount by similar treatment of sub-fraction (6).

Alcohol, $C_6H_{12}O$.—Sub-fractions 1—6 were combined (2·02 g.) and refluxed for 18½ hours with phthalic anhydride (2·3 g.) in pyridine (30 c.c.); the solution was then poured into dilute hydrochloric acid and extracted with ether. The extract was washed with warm 4% sodium carbonate solution and water, dried, and evaporated. The residue was refluxed with sodium ethoxide in benzene (compare following paragraph), and the product, isolated in the usual manner, treated with dry hydrogen chloride. No crystalline hydrochloride could, however, be obtained.

The sodium carbonate solution was acidified, and the acid phthalates, isolated with ether, refluxed with a solution of sodium (1 g.) in alcohol (10 c.c.) and benzene (20 c.c.). The resultant mixture of alcohols (300 mg.) was heated for 3 hours at 110° with excess of freshly distilled pyruvic acid, after which the neutral product, isolated by precipitation with water and extraction with ether, was refluxed with an alcoholic solution of semicarbazide acetate. The *pyruvic ester semicarbazone* was precipitated with water and crystallised repeatedly from benzene–light petroleum, from which it separated in small needles, m. p. (constant) 114—115° (Found : C, 53·35; H, 7·5; N, 17·8. $C_{10}H_{17}O_3N_3$ requires C, 52·9; H, 7·5; N, 18·4%).

Sub-fraction (16).—The colour of this fraction indicated the presence of azulene. It (0·9 g.) was accordingly shaken for 20 hours with a saturated solution of hydroferrocyanic acid (compare Ruhemann and Lewy, *Ber.*, 1927, **60**, 2459). The resultant grey precipitate was washed with ligroin, decomposed with cold 5% sodium hydroxide solution, and extracted with ether. The residue from the dried and evaporated ethereal solution was treated with alcoholic picric acid, whereby a very small amount of a black crystalline solid was obtained, m. p. 115—120° (decomp.); the amount was insufficient for further purification.

Alcohol, $C_{18}H_{36}O$.—Sub-fractions 15, 17—20 were combined and treated with phthalic anhydride by the method already described. The ethereal solution of the product, after removal of pyridine, was washed with warm 4% sodium carbonate solution, but no acid phthalates were extracted by this means, the absence of primary alcohols being thus indicated (Chibnall *et al.*, *loc. cit.*). Aqueous extraction of the ethereal solution, however, removed a considerable quantity of material, which was precipitated by acidification and collected with ether. It was then saponified by heating under reflux for ½ hour with alcoholic sodium ethoxide (50 c.c. of 17%) and benzene (150 c.c.). Water was then added, and the benzene layer washed, dried, and evaporated. The semi-crystalline residue (2 g.) was heated with freshly distilled pyruvic acid (6·2 g.) for 3½ hours at 110—120°, and the neutral product from this reaction converted into the mixed semicarbazones in alcoholic solution. The latter were precipitated with water and repeatedly crystallised from methanol, from which the *pyruvic ester semicarbazone* of the octadecenol separated in rosettes of needles, m. p. 137—137·5° (Found : C, 66·8; H, 10·5; N, 10·5. $C_{22}H_{41}O_3N_3$ requires C, 66·8; H, 10·5; N, 10·6%).

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