CCLXXIX. CRITICAL STUDY OF SHEAR'S ANILINE-HYDROCHLORIC ACID REACTION ASSOCIATED WITH VITAMIN D.

I. FURAN AND DERIVATIVES.

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II. TERPENES.

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I. FURAN AND DERIVATIVES.

SHEAR [1926] reported a test associated with vitamin D made by means of a reagent containing 15 volumes of aniline and 1 volume of concentrated hydrochloric acid. When the test was positive a characteristic red coloration was obtained on heating. The test was given by irradiated cholesterol (containing ergosterol), cod-liver oil and the unsaponifiable portion of cod-liver oil, and by vegetable oils after ultra-violet irradiation.

Shear's reagent is somewhat similar to the aniline acetate reagent for detecting furfuraldehyde. We therefore undertook a study of his reagent with reference to its reactivity with furan and its derivatives. Our tests were carried out by adding 0.1 cc. to 0.2 cc. of the compound if a liquid and 25 to 50 mg. if a solid to 5 cc. of the aniline-hydrochloric acid reagent. The aniline used should be distilled once or twice until it is free from colour. We first observed the reaction at room temperature and then heated to note other changes. The results are given in Table I.

Furan and its derivatives give characteristic colour reactions with the aniline-hydrochloric acid reagent. The colours obtained in the cold vary from red, lavender, purple, green to blue. Furan, tetrahydrofuryl alcohol and tetrahydrofuryl butyrate fail to react without the use of heat. The colours obtained on heating are deep red or brown-red. The colours developed darken on standing. Furoic acid and furylacrylamide do not react at room temperature or after the application of heat.

Thiophen does not react. Pyrrole and some of its derivatives react slightly, but the reactions obtained with these compounds lose their significance in view of the fact that pyrrole when heated with dilute acid alone is known to form a reddish coloration or amorphous red powder. Pyrrole with the aniline-hydrochloric acid reagent gives a reddish brown colour before heating and a deeper brown after heating. The following compounds containing the pyrrole ring do not react in the cold or on heating: indole-3-*n*-propionic acid, skatole, proline, tryptophan and carbazole.

Table I. *Reactions of furan and derivatives with Shear's aniline-hydrochloric acid reagent.*

Compounds	At room temperature	On heating
1. Furan	No change	Deep amber with red-brown tinge
2. Furyl alcohol	Purplish red	Very dark brown
3. Furyl acetate	Brilliant red to lavender to dark lavender	Dark brown
4. Tetrahydrofuryl alcohol	No change	Orange
5. Tetrahydrofuryl acetate	Bright magenta	Deep brown-orange
6. Tetrahydrofuryl butyrate	No change	Yellow-brown
7. Furfuraldehyde	Brilliant purple	Violet precipitate with dark brown liquid
8. Furfuraldehyde acetone	Dark red, deep olive-green	Deep red
9. Furfuraldehyde acetophenone	Deep amber	Mahogany-red
10. Furylacrolein	Green to blue	Red
11. Furylacroleinoxime	Blue to green	Dark brown
12. Furfuraldehyde diacetate	Brilliant red	Mahogany-red
13. Furfuraldoxime	Bright red to purple	Dark brown
14. Hydrofuramide	Bright red to purple	Dark brown
15. Furoin	Brown, light red, dark brown with violet tint	Dark red with violet tint
16. Furil	Yellow-brown to bright red	Dark red-brown
17. Furoic acid	No change; does not dissolve	Dissolves, no change in colour
18. Methyl furoate	Orange-red	Light orange-red
19. Ethyl furoate	Blood colour, solidifies	Dark brown
20. Propyl furoate	Orange-red	Orange-red
21. <i>n</i> -Butyl furoate	Purplish red	Very deep brown
22. Amyl furoate	Deep olive-green	Orange-red
23. Furoyl chloride	Violet to light amber	Amber
24. Furonitrile	Brown-grey to dark brown	Very deep brown
25. Furaerylic acid	Brown-grey to dark brown	Very deep brown
26. Furylacrylamide	No change	No change
27. Furylacrolyl chloride	Black-brown; does not dissolve readily	Black-brown

The Shear reagent also reacts on the application of heat when solid carbohydrates with or without a free carbonyl group are added, yielding greenish brown, yellow-brown, and brown-red colorations. The pentoses, rhamnose, xylose and arabinose, react most vigorously owing to the large amount of furfuraldehyde they are capable of yielding when heated with acid.

II. TERPENES.

Sexton [1928] studied the reagent with reference to sterols and reported negative results with cholestane, cholesteryl acetate and dicholesteryl ether. The compounds that he observed to react positively were the ketone derivatives, and of these the more unsaturated proved the more sensitive. The saturated ketones, cholestane-4-one and cholestane-7-one, proved positive after prolonged standing of the heated reaction mixture, while the unsaturated ketones, cholestenone, oxycholesterol acetate, oxycholestenone and oxycholesterylene, gave intense colour reactions within ten minutes. Two ketones belonging to the terpene series, camphor and carvone, Sexton [1928] also found to give positive reactions. His findings harmonise with those of Heilbron *et al.* [1928], who maintain that vitamin D has ketonic properties. Rosenheim and Webster [1926] regard Shear's reaction as non-specific. They were able to obtain with olive oil after the addition of a trace of benzoyl peroxide or turpentine the same intense reaction as with cod-liver oil.

We have studied Shear's reaction with members of the terpene series, using the method described in Part I (p. 2047). The results are given in Table II.

Table II. *Reactions of terpenes with aniline-hydrochloric acid reagent.*

Compound	Chemical nature	Reaction on heating	Changes on standing after heating
<i>Camphane group</i>			
1. Borneol	Saturated compound; 1 secondary alcohol group	No change	No change
2. <i>d</i> -Camphor	Saturated compound; 1 ketone group	No change	No change
3. <i>dl</i> -Camphor	"	Faint brown-red	Light brown-red
4-6. <i>d</i> -Camphoric acid <i>d</i> -Camphoric anhydride <i>d</i> -Camphorsulphonic acid	"	No change	No change
7. Fenchyl alcohol	Saturated compound; 1 secondary alcohol group	Blood-red	Brown-red
8. Pinene	Unsaturated compound; 1 double bond	Blood-red	Heavy pale brown
<i>Menthane group</i>			
9. <i>p</i> -Menthane	Saturated hydrocarbon	Brown-red	Deep red oil droplets separate out
10. Cineole (eucalyptol)	Saturated compound; 1 bridge oxygen	Light brown	Darker brown
11. <i>i</i> -Menthol	Saturated compound; 1 secondary alcohol group	No change	No change
12. <i>i</i> -Menthone	Saturated compound; 1 ketone group	Dark brown	Dark brown
13. Thujone	Saturated compound; 1 ketone group	Mahogany-red	Mahogany-red
14. Terpene hydrate	Saturated compound; 2 tertiary alcohol groups	Brown-red	Brown-red
15. Terpineol	Unsaturated compound; 1 double bond and 1 tertiary alcohol group	Brown-red	Brown-red
16. Limonene	Unsaturated compound; 2 double bonds	Dark red	Darker red
17. Carvone	Unsaturated compounds; 2 double bonds and 1 ketone group	Brown-red	Deeper brown-red
18. Ionone	Unsaturated compound; 2 double bonds and 1 ketone group	Deep magenta-red; on shaking emerald green observed on sides	Darkens to deep red-brown with disappearance of green colour first observed on sides of tube when shaken
<i>Olefine group</i>			
19. Citronellaldehyde	Unsaturated compound; 1 double bond and 1 aldehyde group	Yellow	Dark brown
20. Citronellol	Unsaturated compound; 1 double bond and 1 alcohol group	Orange-red	Deep brown
21. Isoprene	Unsaturated compound; 2 double bonds	Golden yellow to yellow-brown	Brown
22. Geranial (citraldehyde)	Unsaturated compound; 2 double bonds and 1 aldehyde group	Deep cherry-red	Deep brown-red
23. Geraniol	Unsaturated compound; 2 double bonds and 1 primary alcohol group	Orange-red	Deep brown
24. Linalool	Unsaturated compound; 2 double bonds and 1 tertiary alcohol group	Dark red-brown	Deep red oil droplets separate out

The compounds tested as a rule do not react in the cold. Citral, however, proved to be an exception, giving the colour of intense magenta in the cold. The colour of the reaction mixture at room temperature was slightly darker with terpene hydrate, terpineol and linalool, and slightly lighter with cineole and limonene. Citronellal gave a yellowish white precipitate in the cold, but the characteristic colour reaction was obtained on heating. The colours in the heated mixtures darkened on standing. Red colorations turned deeper red or deep brown.

The aniline-hydrochloric acid reaction is negative with *i*-menthol, borneol, *d*-camphor, *d*-camphoric acid, *d*-camphoric anhydride and *d*-camphorsulphonic

acid; *dl*-camphor reacts very faintly. The reaction is the more intense the greater the saturation of the compound. The olefinic terpenes react most vigorously. The presence of an alcohol, aldehyde or ketone group in an unsaturated terpene serves to intensify the colour reaction. Configuration also influences reactivity. Carvone and ionone are both unsaturated compounds with two double bonds and one ketone group. Yet ionone is far more reactive than carvone.

We cannot confirm Sexton's conclusion that the ketone group is responsible for the Shear reaction. The ketone, *d*-camphor, gives no result and *dl*-camphor (synthetic) gives but a very slight reaction. While the ketones, *l*-menthone, thujone, ionone and carvone, react very strongly, we must not fail to note that many of our most reactive compounds are not ketones.

Carotene yields on heating a deep brown-red colour with Shear's reagent. Carotene is related to the terpenes. Karrer *et al.* [1929; 1930; 1931] subjected β -carotene to permanganate oxidation and obtained β -ionone, a terpene. On ozonising β -carotene he recovered an amount of geronic acid comparable to that obtained from two moles of β -ionone. From α -carotene Karrer obtained amounts of geronic acid comparable with half of that obtained from β -carotene. Heilbron *et al.* [1932] obtained geronic acid as a result of the oxidation of vitamin A. The isolation of this acid confirms the presence of the ionone ring in the structure of the vitamin A molecule. We have examined ionone with the aniline-hydrochloric acid reagent and have found it to be exceedingly reactive.

The Shear reaction is given by substances containing any of the fat-soluble vitamins, by cod-liver oil and cod-liver oil concentrates, by halibut-liver oil or its concentrate and by preparations containing vitamin E. Wheat germ oil, rich in vitamin E, was treated to remove any traces of vitamin A. It was heated at 100° for one hour and oxygen passed through it during the period of heating. The cooled oil gave a stronger reaction with the aniline-hydrochloric acid reagent than the untreated oil.

The Shear reaction is also given by provitamin A (carotene) and by irradiated ergosterol.

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