THE COLOURING CONSTITUENTS OF ALKANET ROOT
(ANCHUSA TINCTORIA, LAM.).


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Alkanet is well known as a red shrub growing in various parts of Europe and Asia. It was known to the Greeks and Romans as Anchusa, is mentioned in the works of Theophrastes and Pliny, and has continued to be regarded as a very valuable product during several centuries past. Its properties and uses are described in the Dictionaries of Chemistry by Watts and Thorpe and also in "The Natural Organic Colouring Matters" by Perkin and Everest.

The most important colouring matter of alkanet root, known as alkannin, has been examined by several chemists, but it is doubtful whether until very recently this dye has been obtained pure. Its composition has been given by various investigators as $C_{17}H_{10}O_4$ (Pelletier, Annalen, 1833, 6, 27), $C_{35}H_{29}O_8$ (Bolley and Wydler, Annalen, 1847 62, 41), $C_{15}H_{14}O_4$ (Carnelutti and Nasini, Ber., 1880, 13, 1514), $C_{15}H_{14}O_4$ or $C_{15}H_{12}O_4$ (Liebermann and Römer, Ber., 1887, 20, 2428) and $C_{39}H_{28}O_8$ (Gawalewski, Chem. Zentr., 1902, 11, 1001; from Zeit. Oesterr. Apoth., 1902, V, 40, 1001). Moreover, according to Liebermann and Römer it was found to give both anthracene and $\beta$-methylanthracene on distillation with zinc-dust. From these results Liebermann and Römer suggested that alkannin might be $\beta$-methyl-1:4(or 1:2)-dihydroxydil(or tetra)hydroanthraquinone, because of the ten known dihydroxyanthraquinones only the 1:4- and 1:2-dihydroxy-derivatives gave either blue or blue-violet colouration with alkali. Carnelutti and Nasini appear to have obtained a diacetyl-alkannin, $C_{19}H_{12}O_4(C_2H_4O)_2$, as a dull yellow micro-crystalline powder, and an indefinite barium compound containing two atoms of barium to three mols. of alkannin. Beyond this no information was available regarding the constitution of alkannin when the present investigation was undertaken.

A representative commercial sample of alkanet root was collected from the Indian bazaars for examination. The petrol extract of the root was subjected to an exhaustive purification, described in detail in the experimental part. Further purification through the acetyl derivative gave a reddish violet micro-crystalline product which was the
purest sample of alkannin that could be obtained, and analysis of this product gave the empirical formula, \( \text{C}_{15}\text{H}_{13}\text{O}_4 \). Several determinations of the molecular weight in camphor by Rast's method (Ber., 1922, 53, 1051) gave varying results, but pointed unmistakably to the molecular formula being double the empirical one. The percentages of barium and lead in the corresponding derivatives also supported the \( \text{C}_{30}\text{H}_{26}\text{O}_8 \) formula for alkannin confirmed by a molecular weight determination of acetylalkannin.

Having thus ascertained the molecular complexity of alkannin, we repeated the acetylation experiment of Carnelutti and Nasini (loc. cit.) and confirmed their result by isolating the acetyl derivative which, however, in the light of our previous observation should be properly represented as a tetra-acetyl derivative of the formula, \( \text{C}_{30}\text{H}_{22}\text{O}_6(\text{C}_2\text{H}_4\text{O})_4 \). Benzylation similarly gave a tetrabenzyol derivative, \( \text{C}_{30}\text{H}_{22}\text{O}_6(\text{CO.C}_6\text{H}_5)_4 \), indicating the presence of four hydroxyl groups in alkannin. The nature of these groups was revealed by methylation, for while methyl iodide did not give definite products, dimethyl sulphate in alkali, which does not generally affect alcoholic groups, gave rise to a dimethoxyalkannin, \( \text{C}_{30}\text{H}_{22}\text{O}_6(\text{OMe})_2 \), which could be benzyolated to a dimethoxydibenzoylalkannin, \( \text{C}_{30}\text{H}_{22}\text{O}_6(\text{OMe})_2((\text{CO.C}_6\text{H}_5)_2 \). These results definitely showed that two of the hydroxyl groups were phenolic, and two alcoholic. It yielded also a dicarbethoxy-derivative, \( \text{C}_{30}\text{H}_{24}\text{O}_6(\text{O.CO}_2\text{Et})_2 \), with ethyl chlorocarbonate. Bromination gave di-, tetra- and hexa-bromo-derivatives, \( \text{C}_{30}\text{H}_{22}\text{O}_6\text{Br}_2, \text{C}_{30}\text{H}_{24}\text{O}_6\text{Br}_4, \text{C}_{30}\text{H}_{22}\text{O}_6\text{Br}_6 \). Bromination of the tetra-acetyl derivative gave tetra-acetyltetrabromoalkannin, \( \text{C}_{30}\text{H}_{22}\text{O}_6\text{Br}_4(\text{CO.CH}_3)_4 \). Alkannin is not oxidised by hydrogen peroxide. Chromic acid and permanganate oxidised it completely and no definite oxidation product could be isolated. Fuming nitric acid also acted very energetically, oxalic and succinic acids being isolated from the mixture together with a nitro-compound, m.p. 287°. Oxidation with dilute nitric acid gave oxalic acid and two derivatives, one containing nitrogen and melting at 297°, the other a nitrogen-free neutral substance, m.p. 320°. Distillation with zinc-dust gave \( \beta \)-methylanthracene. Tetra-acetylalkannin, on treatment with fuming nitric acid, yielded a dinitrotetra-acetylalkannin.

The above results were communicated to the Indian Science Congress in two papers in September 1929 and 1930 and read in the subsequent meetings of the Congress (Proc. Ind. Sci. Cong., 1930, p. 181; 1931, p. 166). Meanwhile we were studying several complex nitro-carboxylic acids obtained from alkannin with dilute nitric acid when papers appeared in the Berichte on the constitution of alkannin. It therefore seems desirable to place the results of our investigation on
record without further delay and reserve the oxidation experiments for a further communication.

Raudnitz, Redlich and Fiedler (Ber., 1931, 64, 1835) purified alkannin from a sample of alkannin reagent supplied by E. Merck by precipitating it from alkaline solution and then repeatedly precipitating it from benzene solution by petrol, and found the empirical formula $C_{16}H_{14}O_4$. They apparently obtained a tetra-acetyl leuco-alkannin, $C_{16}H_{12}O_4(CO.CH_3)_4$, and ascertained the molecular weight in camphor. On distillation in vacuo it gave a small quantity of naphthazarin. Hydrogenation of the acetyl leuco-compound pointed to a side chain with two ethylenic bonds. Basing their arguments on these meagre details they provisionally suggested the following constitution for alkannin,

![Chemical structure of alkannin]

Two months later, however, Dieterle, Salomon and Nosseck (Ber., 1931, 64, 2086) claimed to have obtained pure alkannin corresponding to the formula, $C_{13}H_{14}O_4$, confirmed by the analysis of a diacetate and a dihydroalkannin tetra-acetate. Distillation of alkannin with zinc-dust yielded $\beta$-methylanthracene. Ozonisation in chloroform yielded a compound, $C_{13}H_{8}O_8$, regarded as a dihydroxynaphthaquinonedicarboxylic acid, which lost carbon dioxide on heating. They observed that the behaviour of alkannin towards ozone differed from that of synthetic 5:8-dihydroxy-1-methyl-1:2:3:4-tetrahydroanthraquinone and 5:8-dihydroxy-2-methyl-1:2:3:4-tetrahydroanthraquinone. They therefore concluded that alkannin could not be any of these reduced anthracene derivatives, and that probably its reactions might be best explained by the formula,

![Chemical structure of alkannin according to Dieterle, Salomon and Nosseck]

Raudnitz, however, in a later paper (Ber., 1932, 65, 159) confirms the composition, $C_{16}H_{14}O_4$, by repeating the preparation and analysis of
acetyl leuco-alkannin. He shows that the product of sublimation of alkannin is naphthazarin, not 1-methylquinazarin as supposed by Dieterle, Salomon and Nosseck. He considers it improbable that under the conditions employed by Dieterle, Salomon and Nosseck they obtained 1-methyl- and 2-methyltetrahydroquinazarin.

It is significant that the above investigators do not mention the work of Gawalewski (loc. cit.) who arrived at the formula, \( C_{30}H_{25}O_8 \), for alkannin. It is not probable that Raudnitz, Redlich and Fiedler could obtain pure alkannin by the method they utilized, because we have observed that the waxy material associated with the colouring matter is held so tenaciously that it cannot be removed completely by extracting the dye with alkaline. Moreover the wax and the fatty acids derived from it are all very sparingly soluble in petrol and very rich in carbon, so that even a moderate contamination with any of them may account for the slight increase in the carbon content observed by Raudnitz and his co-workers.

On the other hand, absence of data regarding the molecular weight of alkannin in the experiments of Dieterle, Salomon and Nosseck appears to have caused this confusion regarding its constitution. We consider that a formula satisfactorily explaining the properties of alkannin must embrace the facts that (i) the molecular formula is double the empirical formula, (ii) 3-methylanthracene is one of the degradation products, (iii) naphthazarin and 3-naphthylacetic acid should form part of the skeleton of alkannin, and (iv) the formula should contain two phenolic and two alcoholic groups. It is no doubt very difficult to represent so complex a structure as \( C_{30}H_{25}O_8 \) without careful examination of a greater number of its degradation products, but the results available provide a formula tentatively explaining all the known facts concerning alkannin. We believe that associating a reduced 3-methylanthraquinone with the formula suggested by Dieterle and his co-workers will be the most satisfactory procedure. The production of naphthazarin by vacuum sublimation and of 3-methylanthracene by zinc-dust distillation indicates the following two basic structures in alkannin:

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
\text{OH} & \quad \text{O} \\
\text{OH} & \quad \text{O} \\
\end{align*}
\text{and}
\begin{align*}
\text{O} & \quad \text{CH}_3 \\
\text{O} & \quad \text{O} \\
\end{align*}
\]

The ozonisation experiment of Dieterle, Salomon and Nosseck, yielding a dihydroxynaphthaquinonetricarboxylic acid which becomes
a monocarboxylic acid with loss of carbon dioxide, indicates a side chain of the following type (I) attached to the naphthalene ring

\[ \text{OH} \quad \text{H} - \text{CO}_2\text{H} \]

\[ \text{OH} \quad \text{Me} \cdot \text{CH} : \]

(II)

It may be safely assumed that the two non-phenolic hydroxyl groups exist in the anthraquinone part of the molecule. In accordance with some interesting generalisations made by Mitter (J. Ind. Chem. Soc., 1930, 7, 40) and Mitter and Biswas (ibid., 1928, 5, 769) from a study of numerous anthraquinone derivatives occurring in nature, we suppose that a dihydroxy-\(\beta\)-methylanthraquinone will have one of the hydroxyl groups in the \(\beta\)-position and the other in the \(\alpha\)-position. The structure of the anthraquinone part of alkannin therefore becomes

\[ \text{H}_3\text{C} \quad \text{OH} \quad \text{O} \]

(II)

which accounts for fifteen carbon atoms. If two carbon atoms are added to the side chain of the naphthalene ring (I) we obtain the formula of Dieterle, Salomon and Nosseck (see above) which accounts for the remaining carbon atoms. Two hydrogen atoms will be utilised by a
Combination of the two above formulae which may be represented by a structure of the type

where the positions of the methyl group and the side chain indicated by stars are somewhat uncertain. However, as it happens that in a tetra-substituted anthraquinone derived from (II), the third hydroxyl group takes up the $\alpha$-position we presume that this is the next reactive position and the link with the side chain is established here, possibly by the elimination of this hydroxyl group as water. The detection of traces of succinic acid in the oxidation products of alkannin by Liebermann and Römer and by us, is strong evidence in favour of this conclusion. The alcoholic character of the two hydroxyl groups in the anthraquinone part would be explained if the ring containing them is reduced, so that the molecule of alkannin becomes $C_{30}H_{26}O_8$ and is finally represented by the formula (III)

The possibility of the methyl group in the $\beta$-position being present in the other benzene nucleus necessitating the modification of the above formula as below is likewise excluded from a consideration of the homonuclear arrangement of the hydroxyl groups (cf. Mitter and Biswas, loc. cit.).
This constitution (III) harmonises with the chemical behaviour of alkannin and explains the spectroscopic examination, in which its bands differ so much from the absorption bands given by either naphthazarin or the hydroxyanthraquinones.

It is expected that investigation of degradation products from alkannin, combined with examination of the associated colouring matters in the alkanet root, will throw further light on the constitution of alkannin. Experiments on these lines are in progress.

**EXPERIMENTAL.**

*Extraction of Alkanet Root.*—The powdered root (4 lbs.) was extracted with petrol (b.p. 50-60°) in a modified Soxhlet apparatus (Sando, *Ind. Eng. Chem.*, 1924, **16**, 1125) till the final extract was colourless. The petrol was distilled, the residue dissolved in chloroform was filtered, and the viscous mass left by the chloroform was extracted with cold acetone, in which a white waxy mass remained undissolved. After removing acetone, the residue was repeatedly extracted with 5 per cent. sodium hydroxide solution, which left a blackish violet powder undissolved. After removing acetone, the residue was repeatedly extracted with 3 per cent. sodium hydroxide solution and acidified, the resulting solid being extracted with benzene in a Soxhlet apparatus. On removing the solvent, alkannin remained as a dark-red powder completely soluble in alkali. Yield 1.2-1.5 per cent.

*Tetra-acetylalkannin.*—Alkannin (50 g.) with fused sodium acetate (15 g.) and excess of acetic anhydride was heated under reflux during 5 hours at 140°. The product was poured into excess of water, neutralised with sodium carbonate, and the solid after extraction with
alcohol was crystallised from acetic acid, forming brown micro-crystals. It decomposes at a high temperature, is soluble in ethyl acetate, acetone and acetic acid, but is insoluble in methyl, ethyl and amyl alcohols, ether, benzene etc. Yield 80-85 per cent. (Found: C, 66.5; H, 5.23; COMe, 24.5; M.W. (Rast’s method), 687. C₈₈H₄₄O₁₂ requires C, 66.86; H, 4.99; COMe, 25.2; M.W., 682).

Alkannin.—Tetra-acetylalkannin (50 g.) was hydrolysed with caustic potash (40 g.) in methyl alcohol; poured into water, filtered and acidified. The red flocculent precipitate was dissolved in glacial acetic acid, the solution concentrated, treated while hot with a drop or two of water and allowed to stand. This process was repeated twice, when alkannin separated as a violet-red micro-crystalline powder which shrinks at 180° and decomposes with swelling at 220°; at higher temperatures it becomes a carbonaceous mass (Found: C, 69.74; H, 5.38; M.W. (Rast’s method), 510, 521, 525, 537. C₃₀H₂₆O₈ requires C, 70.0; H, 5.05 per cent. M.W., 514). Yield, 25-30 g.

Methylation.—Alkannin (5 g.) was dissolved in caustic potash (12 g. in 15 c.c. of water) and treated with dimethyl sulphate (15 c.c.) in small quantities with vigorous shaking. The same quantities of methyl sulphate and alkali were again added, the alkaline solution heated under reflux during three hours, acidified, the product again treated with alkali and methyl sulphate as before and finally boiled for an hour and filtered. The solid was washed with hot dilute alkali and water, dried, extracted with chloroform and the solvent distilled. Dimethoxyalkannin separated from dilute acetic acid in brown micro-crystals (Found: C, 70.46; H, 5.85; OCH₃, 12.2. C₃₀H₂₆O₆(OMe)₂ requires C, 70.84; H, 5.53; OMe, 11.44 per cent.). Raudnitz, Redlich and Fiedler failed to methylate alkannin by diazomethane and methyl p-tolylsulphonate.

Benzoylation.—Benzoyl chloride was gradually added to a pyridine solution of alkannin with stirring and cooling; the mixture was then heated on a water-bath during one hour and poured into dilute hydrochloric acid. The sticky mass gradually solidified and separated from alcohol in pale brown micro-crystals. Tetrabenzoylalkannin decomposes above 260° (Found: C, 74.51; H, 4.94. C₃₀H₂₆O₈(CO.C₆H₅)₄ requires C, 74.8; H, 4.52 per cent.). Carnelutti and Nasini (loc. cit.) state that benzoyl chloride does not react with alkannin.

The barium compound.—Alkannin dissolved in absolute alcohol was treated with an ammoniacal solution of barium chloride. The blue precipitate was filtered, and washed thoroughly with water, alcohol and ether (Found: Ba, 12.27. (C₃₀H₂₆O₈)₂Ba requires Ba, 11.78 per cent.).
The lead compound was obtained by adding lead chloride to the solution of the potassium derivative of alkannin, prepared from alcoholic potash and an alcoholic solution of alkannin (Found: Pb, 29.34. \((C_{30}H_{22}O_8)_{2}Pb_2\) requires Pb, 28.75 per cent.).

Dimethoxydibenzoylalkannin.—Dimethoxyalkannin was benzoylated in pyridine, the product after preliminary purification separating from acetic acid in brown micro-crystals (Found: C, 72.9; H, 5.31. \(C_{46}H_{38}O_{10}\) requires C, 73.6; H, 5.07 per cent.).

Dicarbethoxyalkannin.—Alkannin (1 g.) dissolved in pyridine (8 c.c.) was treated with ethyl chlorocarbonate (5 g.) in the cold and the mixture warmed on a water-bath during two hours. On pouring into dilute hydrochloric acid the carbethoxy-compound separated as a blackish brown mass which crystallised from dilute acetic acid in deep brown needles. It does not melt, but decomposes above 270°. The compound can be easily hydrolysed with dilute alkalis (Found C, 65.31; H, 5.42. \(C_{30}H_{24}O_6(OCO_2Et)_{2}\) requires C, 65.6; H, 5.17 per cent.).

Bromination.—Alkannin (2 g.) was placed on a watch glass over which an open dish containing dry bromine (excess) was kept in a desiccator during four days. The viscous product was heated on the water-bath to expel occluded hydrobromic acid, washed with alcohol, dissolved in ethyl acetate and filtered into excess of petrol. This process was repeated three times, the finally violet-brown precipitate separating from acetic acid in deep brick-red micro-crystals. Hexabromoalkannin decomposes on heating (Found: Br, 48.36. \(C_{30}H_{22}O_8Br_6\) requires Br, 48.48 per cent.).

Bromination in cold acetic acid.—Bromine (6 mols.) in acetic acid was added slowly with shaking and under ice-cooling to alkannin (2 g.) dissolved in acetic acid (10 c.c.); after 12 hours at the room temperature, the mixture was poured into water, filtered and washed well with hot water. A portion of the precipitate dissolved in ether. Tetrabromoalkannin was obtained from acetic acid as a brick-red powder blackening at 260° (Found: Br, 38.12. \(C_{30}H_{24}O_8Br_4\) requires Br, 38.46 per cent.). The ether-insoluble portion was dibromoalkannin, crystallising from acetic acid in red aggregates (Found: Br, 23.55. \(C_{30}H_{26}O_8Br_2\) requires Br, 23.74 per cent.).

Bromination in hot acetic acid.—The experiment was conducted as before, but the mixture was heated on a water-bath during three hours and then poured into water. The precipitate, after extraction with ether and alcohol, crystallised from acetic acid in deep brick-red aggregates, and was hexabromoalkannin (Found: Br, 48.21. \(C_{30}H_{22}O_8Br_6\) requires Br, 48.48 per cent.).
Bromination of tetra-acetylalkannin.—The acetyl compound (3 g.) was dissolved in acetic acid (10 c.c.) and shaken with an acetic acid solution of bromine (4 mols.) added in small quantities at a time. The mixture was heated on a water-bath during one hour, cooled and poured into water, when a brownish precipitate separated. After extraction with carbon disulphide the residue was dissolved in acetic acid from which one drop of water precipitated tetra-acetyltetrabromoalkannin as a brown, granular mass. For complete purification, the above process had to be repeated three times. The compound decomposes at about 280° with blackening (Found : Br, 31.82. \( \text{C}_3\text{H}_2\text{O}_8\text{Br}_4(\text{C}_2\text{H}_4\text{O})_4 \) requires Br, 32.0 per cent.).

Oxidation with fuming nitric acid.—Alkannin (5 g.) was gradually added to nitric acid (15 c.c., d. 1.5) at 0°. After an hour’s stirring the mixture was allowed slowly to attain the room-temperature when oxides of nitrogen were freely evolved. Action having ceased, the mixture was poured on crushed ice, filtered and washed well with ice-water which dissolved a large proportion. The insoluble residue separated from alcohol in reddish brown micro-crystals, m.p. 287° with explosion; it contains nitrogen and is under investigation. The filtrate on evaporation to dryness deposited a pasty mass which on extraction with ether left a powder crystallising from alcohol in colourless prismatic needles identified as oxalic acid (m.p. 101°). No definite compound could be isolated from the ether extract.

Oxidation with dilute nitric acid.—Alkannin (5 g.) was added to nitric acid (100 c.c., d. 1.2) shaken well during one hour and then slowly heated on a water-bath during 48 hours, fairly vigorous action with frothing taking place at first; after 24 hours the solution became yellow. Dilution gave an orange nitro-compound which separated from acetic acid in orange-yellow prismatic crystals, m.p. 297° (decomp.). The filtrate on evaporation and extraction with ether left a white powder which was washed with alkali; it crystallised from alcohol in colourless needles, m.p. 320°, and did not contain nitrogen, while oxalic acid was isolated from the alkaline solution. The ethereal extract was evaporated, and a solution of the residue in alcohol was boiled with animal charcoal; from the filtrate when kept in a vacuum during several days there separated a few crystals which on recrystallisation melted at 178°, corresponding nearly to succinic acid.

Action of fuming nitric acid on tetra-acetylalkannin.—Tetra-acetylalkannin (2 g.) dissolved in acetic anhydride (5 c.c.) was gradually added to a mixture of nitric acid (3 c.c., d. 1.5) and acetic anhydride (8 c.c.) under cooling. After 12 hours the mixture was poured into water, the resulting reddish brown solid separating from acetic acid as a brown micro-crystalline powder. The dinitrotetra-acetylalkannin does
not melt, but blackens at 260° (Found: C, 59.12; H, 4.61; N, 3.38. \( \text{C}_{32}\text{H}_{32}\text{O}_{16}\text{N}_{2} \) requires C, 59.07; H, 4.15; N, 3.62 per cent.).

Distillation of alkannin with zinc-dust.—A mixture of alkannin and zinc-dust in equal quantities was distilled under reduced pressure, yielding a yellowish oil which solidified slowly; the product was dissolved in benzene, washed with sodium carbonate and hydroxide solutions and freed from solvent. The residue was extracted with alcohol which on concentration gave a few crystals of \( \beta \)-methylandanthracene, m.p. 198-200°.

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