116. *Calycanthine.*

By (the late) G. BARGER, JUAN MADINAVEITIA, and PAUL STREULI.

This alkaloid has the formula $C_{22}H_{26}N_4$, not $C_{22}H_{28}N_4$ as previously supposed. By a variety of reactions it yields a stable substance, *calycanine*, $C_{16}H_{10}N_2$. Nitric acid converts calycanthine into two *substances*, probably tetranitrocalycanine-mono- and -di-carboxylic acid. When the alkaloid is heated with soda-lime at 305°, nearly half its weight of *N*-methyltryptamine is obtained; benzoylcalycanthine gives instead a little 2-phenylindole. When heated with calcium oxide, calycanthine yields a fluorescent base, $C_{12}H_{10}N_2$, probably a methyl-4-carboline.

THE formula $C_{11}H_{14}N_2$ assigned to the alkaloid by Gordin (*J. Amer. Chem. Soc.*, 1905, **27**, 144, 1418; 1909, **31**, 1305; 1911, **33**, 1626) was doubled by Späth and Stroh (*Ber.*, 1925, **58**, 2131). The double formula, which had already been conceived in 1919 by one of us from molecular-weight determinations, was moreover indicated in Gordin's last paper by a peculiar methiodide, to which he assigned the formula $C_{24}H_{28}ON_3I,H_2O$. Hence, although calycanthine gives Hopkins and Cole's reaction for tryptophan, it is not a simple methyltryptamine, as the single formula would suggest. That it is nevertheless derived from this amine was shown by Manske (*Canadian J. Res.*, 1931, **4**, 275), who obtained benzoyl-*N*-methyltryptamine by oxidising benzoylcalycanthine with permanganate. Manske (*J. Amer. Chem. Soc.*, 1929, **51**, 1836) further discovered calycanthine in the seeds of *Meratia praecox*, a Composite; the occurrence of the same alkaloid in two unrelated natural orders (*Calycanthaceæ* and *Compositæ*) may perhaps suggest a simple biogenesis. Thus harman and its derivatives are found in various orders, but the complicated process which for instance leads to the formation of strychnine from tryptophan seems only to have been evolved on one particular twig of the genealogical tree.

The micro-analysis of calycanthine presents great difficulty in so far as the carbon content was always found about 2% low. Since there is no oxygen, however, the carbon content can be deduced by difference and we are confident that the formula is $C_{22}H_{26}N_4$

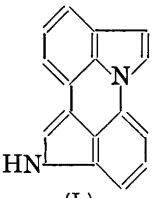
(that of Späth and Stroh, with two more hydrogen atoms, was obtained by doubling Gordin's formula, without further carbon and hydrogen determinations). Gordin found 1 (0·88) methylimino-group for $C_{11}H_{14}N_2$ and we find 1·0 and 1·05 for the doubled formula; this group belongs to the *N*-methyltryptamine moiety. Späth and Stroh found 1·57 equivalents of active hydrogen; Dr. H. Roth found for us 2·0 and 2·1 in pyridine at 22°, but 3·7 and 3·9 at 95°. This effect of temperature we have encountered in another indole alkaloid, aspidospermine, which gives 0 and 1 equivalent at 22° and 95° respectively. We are unable to explain the four hydrogen atoms in calycanthine apparently mobilised at 95°, but the two mobilised at room temperature agree with the nitrosoamine of Gordin and the dibenzoyl derivative obtained by Manske. One of these two active hydrogen atoms is in the *N*-methyltryptamine portion of the molecule; the position of the other remains doubtful. We find no *C*-methyl groups.

Numerous oxidation experiments gave little information about the constitution. We therefore had recourse to rather unorthodox methods involving a high temperature; on heating to 305° with soda-lime, we obtained 45% of the calycanthine as *N*-methyltryptamine, $C_{11}H_{14}N_2$, and with calcium oxide a much smaller yield of a *base*, $C_{12}H_{10}N_2$, which appears to be a methyl-4-carboline, isomeric with harman. It seems unlikely that this base arises from methyltryptamine by a secondary reaction, and we are inclined to think that it represents the second moiety of the molecule and that its twelfth carbon atom alone is derived from the tryptamine portion, which, under optimal conditions, is set free as such in a yield of 90% of the theoretical by soda-lime, but only in much smaller yield by calcium oxide. The main effect of the latter reagent is to bring about various fissions, in one of which one carbon atom of the tryptamine moiety remains attached to the carboline moiety of the molecule. Most indole alkaloids are derived from a single tryptamine residue, and contain two nitrogen atoms. In evodiamine and rutaecarpine, with three nitrogen atoms, we may assume two tryptamine residues, of which the second is broken down to formylanthranilic acid, supplying the third nitrogen atom. Calycanthine with four nitrogen atoms would seem to be derived from two molecules of tryptamine, without loss of nitrogen; both tryptamine units have acquired an additional carbon atom (from formaldehyde), leading, without ring closure, to *N*-methyltryptamine, and, with ring closure, to a 4-carboline. If this hypothesis is correct, the main problem is to determine how the two moieties are united. $C_{11}H_{14}N_2 + C_{11}H_8N_2$ would make $C_{22}H_{22}N_4$ and for each bond between the two halves there would be two hydrogen atoms less. Since calycanthine contains twenty-six hydrogen atoms, there must be hydrogenation of one or more rings.

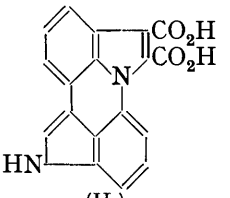
A number of degradation products of calycanthine are evidently derived from parts of both moieties. Chief of these is a feeble base, $C_{16}H_{10}N_2$, formed in small yield by a variety of reagents (see p. 515) and having altogether extraordinary stability: we suggest for it the name *calycanine*. It also results, still more significantly, by treatment of calycanthine by chromic acid in boiling acetic acid solution. Boiling concentrated nitric acid oxidises the alkaloid to an *acid*, $C_{17}H_6O_{10}N_6$, apparently tetranitrocalycaninecarboxylic acid, and dilute nitric acid at 150° similarly to an *acid*, $C_{18}H_6O_{12}N_6$, presumably the corresponding dicarboxylic acid. The conversion of these acids into calycanine has so far proved impossible. Calycanine would appear to be built up of the indole nuclei of both moieties of the alkaloid. The methylamino-group from the tryptamine side chain cannot enter into its constitution, and it is difficult to involve the pyridine ring of the carboline. Calycanine contains one active hydrogen atom and hence one imino-group. The other nitrogen is probably attached to three different carbon atoms, as already suggested by Manske. Two indole molecules would have a total of fourteen hydrogen atoms; calycanine has ten, so the two molecules would be joined by two bonds constituting an additional ring; these two bonds should involve the nitrogen of one half, but not of the other. In this way we arrive at (I) for calycanine, and the last-named oxidation product of calycanthine with nitric acid can readily be formulated as (II). There are of course many isomerides $C_{16}H_{10}N_2$ and we merely put forward (I) tentatively to facilitate discussion. It represents a di-indolylene, but also a quinoline derivative, which latter would be in accordance with its feebly basic nature; calycanine dissolves in dilute mineral acids and in moderately concentrated

acetic acid with a yellow colour, which disappears on neutralisation. The stability of calycanine may be illustrated by the experiment described on p. 514.

Some additional support for (I) is supplied by the formation of quinoline and 2-phenylindole when benzoylcalycanthine is heated with soda-lime to 300° (calycanthine itself under these conditions forms *N*-methyltryptamine). It might be argued that the introduced



(I.)



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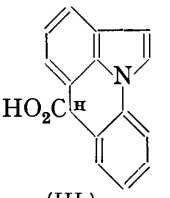
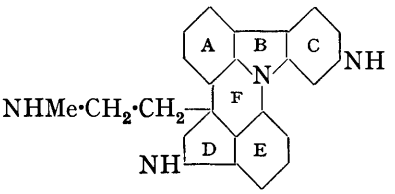
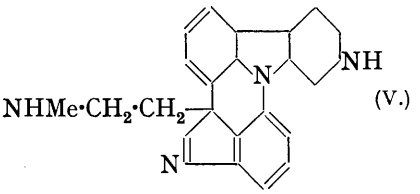


(III.)

benzoyl group furnishes the quinoline, but since benzoyl-*N*-methyltryptamine survives heating with calcium oxide to 300°, it seems more likely that the benzoyl group is removed by soda-lime and the resulting benzoic acid is converted into benzene. It is likewise improbable that the introduced benzoyl group contributes to the formation of 2-phenylindole. Although (I) does not allow for the direct formation of the 2-derivative, this is known to be formed readily from 3-phenylindole, the formation of which, and of 1-phenylindole (also isomerised), is readily explicable by (I). In calycanine two indole nuclei are united by two bonds, and it might be questioned whether one of these (C–C) is not formed by a secondary dehydrogenation, and is absent from the alkaloid itself; we think such a joining of a benzene with a pyrrole ring at 120° in the presence of chromic acid unlikely. The great stability and volatility of calycanthine are entirely in accordance with a condensed ring system, as in (I). In order to complete the carbon skeleton of calycanthine we must add to the tryptamine residue its side chain and to the carboline moiety its pyridine ring. The latter ring can only be added to that indole complex in (I) which has both its 2- and its 3-position free, and thus we obtain (IV) as an expression for the carbon skeleton

(IV.)




(V.)

of completely hydrogenated calycanthine, $C_{22}H_{36}N_4$, in which six double bond will have to be introduced. Most of these will be in D and E; E in particular is probably aromatic, on account of the large yield of methyltryptamine. A, B, and C on the other hand seem more strongly hydrogenated. There are several reasons for believing that this is the case with ring C. In the first place, although on heating calycanthine with calcium oxide at 300° a fluorescent *base*, $C_{12}H_{10}N_2$, is produced, there was obtained at 260° a small quantity of a *base*, $C_{12}H_{16}N_2$ (or $C_{12}H_{18}N_2$), which does not fluoresce and seems to be a hydroharman derivative. In the second place, calycanthine gives with *p*-dimethylaminobenzaldehyde in the cold at most a pale yellow colour; on heating, a claret colour is developed, which again fades on cooling. This reversible colour change can be repeated indefinitely and is given by 2 : 3-dimethylindole, and by tetrahydroharmine (Perkin and Robinson, J., **1919**, **115**, 939). It is also given by tetrahydroharman, *N*-methyltetrahydroharman, and by calycanthidine, a simple alkaloid, $C_{13}H_{16}N_2$, which accompanies calycanthine (Barger, Jacob, and Madinaveitia, *Rec. Trav. chim.*, **1938**, **57**, 548). Hence it would seem that calycanthine also contains a reduced pyridine ring. Ring A on the other hand is probably not hydrogenated, for calycanthine (like calycanthidine) couples with diazotised *p*-nitroaniline to form a deep red dye similar to that obtained by Perkin and Robinson with tetrahydroharmine. If there is an imino-group in ring C it would be the second basic group of the molecule, and the nitrogen in D must be tertiary, since there are only two active hydrogen atoms. This places four double bonds in D and E, and leaves only

two for A as shown in (V), which formula is merely given as an example of the difficulty of allocating the double bonds. Since the methylcarboline produced by quick-lime has an active hydrogen atom, it cannot be 1-methyl-4-carboline, which moreover has a much lower melting point (Spath and Lederer, *Ber.*, 1930, **63**, 2110); nor is it identical with harman. If the carbon skeleton of (IV) is correct, the fission product might be 12-methyl-4-carboline, with the methyl group derived from the quaternary carbon atom of D. This is being tested by synthesis.

The quaternary β-carbon atom of an indole nucleus in D is not without analogy in Nature, for it occurs in physostigmine, to which calycanthine shows some analogy. The tryptamine side chain in (IV) and (V) is angular and is readily lost by pyrolysis, leading to (I). If, on the other hand, the bond between this quaternary atom and A is ruptured (along with that between B and E), *N*-methyltryptamine would be set free. This fission is brought about by soda-lime, but not by quick-lime, and is evidently a hydrolysis rather than a pyrolysis. We are however at a loss to understand why among the various degradation products of calycanthine there is not one from which only the side chain has been lost, so that all six rings A—F remain together. Ring C has so far only survived, when D, E, and F were destroyed. Manske considers that the side chain of the *N*-methyltryptamine does not occur as such in the anhydrous alkaloid, but is present as part of a carboline complex, one ring of which is opened by benzoylation. He assumes (as we do) that one nitrogen atom is a member of three rings, but suggests that the 3-carbon atom of the unstable carboline is joined to the rest of the molecules, whereas we have considered above that the second junction between the two halves of the molecule is made through the 12-carbon atom of a more stable carboline complex. If the same two nitrogen atoms are involved in the production both of *N*-methyltryptamine and of our fluorescent base $C_{12}H_{10}N_2$, then the ideas which have led us to suggest (IV) and (V) require considerable modification and half the molecule still remains unaccounted for. We are unable to disprove Manske's assumption, but would point out that, if the methylamino-group of calycanthine is not free at the end of a side chain, it becomes so with extraordinary ease, for when the alkaloid is heated with methyl iodide, in the absence of any other solvent, one nitrogen atom is lost as a volatile amine. This also happens to some extent when *N*-methyltryptamine itself is heated with methyl iodide under similar conditions, as a model experiment has shown.

The methylation of calycanthine is at present being investigated; it proceeds with considerable difficulty, as was pointed out by Späth and Stroh, and in an anomalous manner, as already described by Gordin. Using a variety of experimental conditions and reagents, we have not yet been able to isolate a normal dimethiodide such as the substance, m. p. 261—262°, mentioned by Späth and Stroh, with an iodine content corresponding to $C_{22}H_{24}N_2Me_2,2MeI$. A considerable portion of the alkaloid always escapes methylation and is converted into the dihydriodide and also the *monohydriodide*, $C_{22}H_{26}N_2,HI$, m. p. 260°. The only methylation products we have obtained contain N_3I; one, obtained by heating calycanthine with methyl iodide alone, is naturally free from oxygen, ($C_{21}H_{22}N_3I$?), although its melting point corresponds to that of Gordin's substance $C_{24}H_{30}ON_3I$, m. p. 325°. When methyl alcohol is present, there is formed in addition a substance $C_{22}H_{26}ON_3I$, H_2O, m. p. 240—242°, which, if it contains a methoxy-group at all, is very resistant to hydrolysis. Hence we are unable to trace an analogy to gramine (Madinaveitia, J., 1937, 1927; cf. Jacob and Madinaveitia, *ibid.*, p. 1929) or to carbolines (Perkin and Robinson, J., 1919, **115**, 946; Barger, Jacob, and Madinaveitia, *Rec. Trav. chim.*, 1938, **57**, 549). The nitrogen atom is not lost in the form of ammonia, as Gordin supposed, but as a volatile amine; in other respects we confirm his description. This peculiar behaviour of calycanthine may be connected with the mobilisation of four hydrogen atoms by pyridine and methylmagnesium iodide at 95° (see above). We have endeavoured to prove the existence of a tryptamine side chain by heating the alkaloid with acetaldehyde at p_H 5, in the hope of producing an additional ring and a second carboline group (cf. Akabori and Saito, *Ber.*, 1930, **63**, 2245). The product of the reaction, an oily base, responded to this hope in so far as it did not give the Hopkins–Cole reaction, or that of Ehrlich, indicating that an α-position in a pyrrole ring had been substituted. The new base was analysed as its *dipicrate*; only a few mg. were available, so its formula is doubtful, but it seems that condensation with acetaldehyde had taken place.

Numerous attempts, other than those already mentioned, to oxidise or dehydrogenate calycanthine have so far not yielded pure products; *e.g.*, with ozone, mercuric and silver acetates, palladium and maleic anhydride or safrole, permanganate in neutral solution and in glacial acetic acid, manganese dioxide and sulphuric acid, mercuric sulphate and sulphuric acid, lead oxide in glacial acetic acid, Caro's acid. The resistance of this oxygen-free alkaloid to some reagents is quite extraordinary; *e.g.*, it was recovered unchanged after the action of nascent oxygen from bleaching powder and cobalt nitrate in boiling solution; it was hardly attacked by potassium hydroxide in boiling amyl alcohol, although the flask was devitrified; it sublimed out of fused potassium hydroxide. Drastic methods of degradation, which we have largely employed so far, are open to criticism, yet similar ones led to the recognition of the carbon skeleton of another indole alkaloid, yohimbine (Mendlik and Wibaut, *Rec. Trav. chim.*, 1931, **50**, 91; Barger and Scholz, *Helv. Chim. Acta*, 1933, **16**, 1343). In spite of its small yield, Diels's hydrocarbon was a key to the constitution of cholesterol.

Experimental.

The powdered achenes of *C. floridus* (45 kg.) were defatted with light petroleum and then percolated with alcohol. We are much indebted to Dr. J. J. Blackie of Messrs. Duncan, Flockhart and Co., Edinburgh, for having done this for us. The alcoholic extract, reduced to 3 l., was mixed with 1 l. of water and 100 c.c. of hydrochloric acid, washed with ether, and basified with sodium hydroxide. The white precipitate became crystalline in a few minutes; it was boiled in acetone solution with 0·2 g. of charcoal and water was added to the filtrate until a turbidity appeared; on cooling, large crystals separated. After recrystallisation, and concentration of the combined acetone–water mother-liquors, the final crop was separated by repeated crystallisation from alcohol into the more soluble crude calycanthidine (Barger, Jacob, and Madinaveitia, *loc. cit.*) (yield, 0·03% of the seeds) and pure calycanthine (yield, 0·64%). The latter formed octahedra, m. p. 216°. After prolonged heating at 120° in a high vacuum over phosphoric oxide, or after recrystallisation from anhydrous benzene, the m. p. was raised to 242° (Gordin, 243—244°; Späth and Stroh, 245°). Gordin attributed the rise to the loss of $\frac{1}{2}$ H_2O (on the doubled formula, $1H_2O$); we have not observed a loss of weight corresponding to this.

For analysis the base was crystallised several times from dry acetone and absolute alcohol, and dried as above to constant weight (Found: C, 75·0, 75·1, 74·5, 73·8, 74·0, 73·7, 74·9, 74·9, 74·8, mean 74·5; H, 7·47, 7·65, 7·61, 7·68, 7·66, 7·53, 7·44, 7·55, 7·66, mean 7·58; N, 15·95, 15·75, 16·04, 16·05, 16·22, 16·15, mean 16·03. Calc. for $C_{22}H_{26}N_4$: C, 76·26; H, 7·57; N, 16·18%. Calc. for $C_{22}H_{28}N_4$: C, 75·82; H, 8·10; N, 16·08%). The figures obtained by three expert micro-analysts agree as to H and N, but not as to C; the total for the three elements amounts to 98%. We therefore submitted the question to Dr. H. Roth of Heidelberg, who, in editing the 4th edition of Pregl's book, had stated that no substance had proved insusceptible of micro-analysis. He very kindly did some additional combustions with special precautions, without, however, finding more C; he confirmed the first four (Dumas) nitrogen determinations by the micro-Kjeldahl method (Found: 16·22, 16·15). We are greatly indebted to him for his help in this matter. If we calculate the carbon by difference, there is a close agreement with $C_{22}H_{26}N_4$ and we think that $C_{22}H_{28}N_4$ may be excluded.

Calycanine.—Finely powdered calycanthine (0·1 g.), mixed with zinc dust (3 g.), was heated between asbestos plugs in a Pyrex tube, 0·5 × 15 cm. The distillate at both ends soon partly crystallised; the crystals were washed with a little acetone, and repeatedly sublimed in a vacuum (bath 180—200°). The product formed silky needles, m. p. 296—297°, from pyridine; a specimen repeatedly sublimed at atmospheric pressure over soda-lime had m. p. 310° and was also analytically pure (Found: C, 83·3, 83·3; H, 4·35, 4·45; N, 12·4, 12·2. $C_{16}H_{10}N_2$ requires C, 83·5; H, 4·4; N, 12·2%. Active hydrogen found: 0·91 equiv. at 23°, 0·95 at 95°). The acetone washings of the sublimate yielded an oily residue, from which acid extracted unchanged calycanthine, identified after vacuum sublimation. The yield of *calycanine* was only 30—40 mg. per g. of calycanthine used (not counting the alkaloid recovered from the mother-liquors).

Calycanine sublimes without decomposition slightly below 300° at atmospheric pressure. It also sublimes unchanged over soda-lime, and is only very slightly attacked by prolonged heating with soda-lime at 350° (see below). A sample evaporated three times on the water-bath with fuming nitric acid was recovered without appreciable decomposition. The substance is a feeble base; it is not soluble in dilute acetic acid, and if dissolved in a more concentrated acid, can be extracted by ether. Since the salts are so readily hydrolysed, none could be isolated.

Calycanine is little soluble in ether and acetone, slightly more in methyl alcohol, more readily in pyridine. Its formation by zinc dust distillation seems to be more an effect of temperature than of the zinc. Thus, when 0·2 g. of calycanthine was heated in an open test-tube in a metal-bath, a liquid distillate began to condense at 250°, and at 280—300° a sublimate of calycanthine appeared. Extraction of the total distillate with a little ether left a small crystalline residue of calycanine; from the extract, at least one third of the calycanthine was recovered unchanged.

Since calycanine thus results from pyrolysis, it is formed by heating calycanthine with a variety of agents: red lead oxide, copper oxide wire, selenium, sulphur. The yield was about 5% of the theoretical and less in the case of selenium and sulphur. After heating with copper oxide for 3 hours at 320° some calycanthine was recovered unchanged. The best yield of calycanine (10%) was obtained with palladium-black (50 mg. of calycanthine, 100 mg. of palladium, 12 hours at 200—220°): a sublimate of calycanine formed and no calycanthine or other substance could be isolated by extraction of the palladium. Calycanine was obtained at a still lower temperature by the dehydrogenation of calycanthine with chromic acid. To calycanthine (10 g.) in 100 c.c. of glacial acetic acid, a solution of chromic acid (10 g.) in 15 c.c. of water + 50 c.c. of glacial acetic acid was slowly added; an initial precipitate of calycanthine chromate dissolved after a slight rise of temperature, which was kept below 25° by cooling. The solution was refluxed for 5 hours and chromic acid could then not be detected by hydrogen peroxide. The cooled solution was diluted with water and extracted ten times with ether. The extract was washed with sodium carbonate solution, dried, and concentrated to a small bulk. The crystals that separated were twice recrystallised from methyl alcohol, sublimed in a high vacuum at 200°, and crystallised from pyridine; m. p. 295—298° (Found: C, 83·2; H, 4·4; N, 11·8%). The chromic acid solution contained a nitrogenous acid.

Oxidation of Calycanthine with Nitric Acid.—Concentrated nitric acid (100 c.c.) was added to 0·2 g. of calycanthine, the first few drops slowly until the solid liquefied. The dark green solution formed, after refluxing for 12 hours, became pale yellow and crystals separated. After evaporation at atmospheric pressure to a small volume, the solution was treated with 25 c.c. of fuming nitric acid and again refluxed for 12 hours. On cooling, well-formed needles separated, which slowly decomposed at 330—340° without melting. The *product* (tetranitrocalycanine-monocarboxylic acid ?) could only be recrystallised from concentrated nitric acid or nitrobenzene owing to its sparing solubility in most solvents (Found: C, 44·0, 43·5; H, 1·5, 1·5; N, 18·8, 19·3. $C_{17}H_6O_{10}N_6$ requires C, 44·9; H, 1·3; N, 18·5%. $C_{16}H_6O_{10}N_6$ requires C, 43·4; H, 1·36; N, 19·0%). It was only very slightly soluble in cold aqueous sodium carbonate or hydroxide, but more soluble on heating, to a red solution. The very sparingly soluble sodium salt crystallised on cooling in long, red, hair-like needles, which contained approximately the amount of sodium required for a monocarboxylic acid; on heating with lime, carbon dioxide was evolved.

A second oxidation *product* (tetranitrocalycaninedicarboxylic acid ?) was obtained by heating calycanthine with 30% nitric acid at 150° in a sealed tube. It formed yellow crystals, practically insoluble in cold sodium carbonate solution, but slightly soluble with an orange colour on heating (Found: C, 43·8; H, 1·9; N, 17·0. $C_{18}H_8O_{12}N_6$ requires C, 43·2; H, 1·6; N, 16·8%. $C_{18}H_6O_{12}N_6$ requires C, 43·4; H, 1·2; N, 16·9%).

Methylation of Calycanthine (Preliminary Experiments).—When calycanthine was refluxed with methyl iodide and methyl alcohol, a mixture of at least four substances was formed: (a) calycanthine dihydriodide, already described by Gordin; after drying in a high vacuum at 15°, it had the composition $C_{22}H_{26}N_4,2HI,H_2O$ and melted at 218—219° after sintering at 215°. On drying in a high vacuum at 100° it became anhydrous and then melted at 226—227°. (b) *Calycanthine monohydriodide*, m. p. 260° (Found: C, 55·6; H, 5·7; N, 11·9; I, 27·8. $C_{22}H_{26}N_4,HI$ requires C, 55·7; H, 5·7; N, 11·9; I, 26·8%). (c) An oxygenated quaternary *salt*, forming long needles, m. p. 240—242°, from water. Heated at 150° in a current of dry air, it did not lose weight (Found: C, 53·8, 54·0, 53·3, 53·3; H, 5·6, 5·8, 5·4, 5·6; N, 8·5, 8·6; I, 25·9, 25·7. $C_{22}H_{28}O_2N_3I$ requires C, 53·55; H, 5·7; N, 8·5; I, 25·8%). Heated in a high vacuum at 100°, this salt lost 4·2% ($C_{22}H_{26}ON_3I,H_2O$ requires H_2O, 3·7%). (d) An oxygen-free quaternary *salt*, m. p. 317—318°, or 325° (corr.), more soluble in water than (c) (Found for the substance dried at 100°: C, 57·0; H, 5·2; N, 9·5. $C_{21}H_{22}N_3I$ requires C, 56·9; H, 5·0; N, 9·5%. Found for the air-dried substance: N, 8·5, 8·5; I, 25·7. $C_{21}H_{22}N_3I,3H_2O$ requires N, 8·5; I, 25·6%). The determination of methoxy-groups in (c) and (d) yielded only very small amounts of silver iodide (Found respectively 1·5 and 1·4%. 1MeO would require 6·3 and 6·2%). Since (d) when anhydrous is oxygen-free, it cannot contain a methoxy-group. In both cases a little *N*-methyl is probably liberated (from an indole nitrogen atom ?). It is conceivable, that (c) and (d) in the anhydrous condition differ only by a molecule of methanol of crystallisation.

Action of Acetaldehyde.—Calycanthine (0·5 g.) was heated in dilute sulphuric acid (p_H 5) in a sealed tube with excess of acetaldehyde for 2 hours at 130°. On basification and extraction with ether, an oily base was obtained, b. p. 220°/1 mm., which gave no colour reactions with the Ehrlich and the Hopkins–Cole reagent and yielded a crystalline *dipicrate*, m. p. 222° (Found: C, 51·9; H, 4·5; N, 15·8. $C_{24}H_{28}N_4,2C_6H_3O_7N_3$ requires C, 52·0; H, 4·1; N, 16·9%).

Action of Soda-lime on Calycanthine.—The alkaloid (1 g.), mixed with finely powered soda-lime (15 g.), was heated in a sealed tube at 300—320° for 15 hours. There was always some pressure, and formation of methylamine. The product was extracted with ether, and the extract shaken successively with hydrochloric acid, sodium hydroxide solution, and water; on drying and evaporation, only a trace of a dark neutral resin remained. Nearly all the material had passed into the hydrochloric acid, and after this solution had been basified, ether extracted a resinous oil, which partly distilled at 220—230°/ 15 mm. The distillate solidified at 0°; after recrystallisation from ligroin it had m. p. 86°, not depressed by authentic *N*-methyltryptamine. The identification was confirmed by colour reactions (Ehrlich; glyoxylic acid) and by analysis (Found: C, 76·3; H, 8·0; N, 16·0. Calc. for $C_{11}H_{14}N_2$: C, 75·9; H, 8·0; N, 16·1%). The maximal yield was 0·22 g. from 0·5 g. of calycanthine or 88% of the theoretical from one moiety of the molecule; the other moiety had been converted into non-volatile bases and volatile substances which escaped identification. The high yield was only obtained after the optimal conditions had been found by numerous experiments. For example, calycanthine was not appreciably attacked below 280°, and a considerable portion remained unchanged after 15—20 hours at 290°; at 330°, *N*-methyltryptamine was itself broken down to simpler indole derivatives which then readily polymerised. Apart from the temperature the composition of the soda-lime had a great influence on the yield; the different behaviour of various specimens led us to dry the reagent to constant weight on the water-bath in a vacuum over phosphoric oxide.

Action of Soda-lime on Benzoylcalycanthine.—The crude substance, prepared from 1 g. of pure calycanthine by Manske's method, was dissolved in chloroform. The solution was poured on 15 g. of dry soda-lime in a tube, and evaporated so as to secure an intimate mixture. After 15 hours at 305° a considerable pressure and the odours of quinoline and of indole were observed. The product was treated as described in the previous section.

Neutral fraction. On distillation, two fractions were obtained, (a) b. p. 110°/15 mm., (b) b. p. 240—250°/15 mm. The former was an odourless *oil*, which gave with *p*-dimethylaminobenzaldehyde a coloration about three times as intense as that obtained with indole; the oil also gave the glyoxylic reaction. It crystallised in the ice-chest and melted at + 2°. On exposure to air it decomposed in a few hours and then smelt of indole (Found: C, 81·0; H, 7·4; N, 8·8; active H, 0·61. $C_{11}H_{11}N$ requires C, 84·0; H, 7·0; N, 8·9; 1 active H, 0·63%). We are unable to account for the deficit of carbon, which is also shown by calycanthine itself (see above); the presence of one oxygen atom in the distilled and crystallised substance would require a molecular weight incompatible with the low boiling point. Fraction (b) was 2-phenylindole; it crystallised at once in the receiver and formed aggregates of needles (from ligroin), m. p. 186°. At 170° in a high vacuum the substance sublimed in large crystals (Found: C, 86·9; H, 5·6; N, 7·2; active H, 0·45. Calc. for $C_{14}H_{11}N$: C, 87·0; H, 5·7; N, 7·2; 1 active H, 0·56%). The substance gave a brilliant pink Ehrlich reaction, soon changing to pure blue; its m. p. was not depressed by authentic 2-phenylindole. The maximal yield was only 10 mg. from the benzoylation product of 1 g. of calycanthine. A change in the hydration of the soda-lime, or of 10° in the temperature of the reaction, or even in the diameter of the sealed tubes, prevented the formation of sufficient 2-phenylindole for isolation (which was sometimes made difficult by resinous degradation products).

Basic fraction. This mainly distilled at 90—120°/15 mm. and consisted largely of quinoline; a small, less volatile portion could not be purified. On redistillation a little was collected at 90°/15 mm. and yielded a picrate with C and H content equal to that of quinoline picrate, but a somewhat depressed m. p. (194°). Since it seemed impossible to separate the basic fraction into its constituents by distillation, it was converted into picrates, which were fractionally crystallised from acetone. The least soluble crops (m. p. 200°; C, 50·6; H, 2·9%; m. p. 196°) could not be identified with either quinoline or *iso*quinoline picrate, of which they lowered the m. p.'s, but the main crop, of more soluble picrate, was found identical with that of quinoline; m. p. 198° after crystallisation from alcohol (Found: C, 50·1; H, 3·0; N, 15·5. Calc. for $C_9H_7N,C_6H_3O_7N_3$: C, 50·3; H, 2·8; N, 15·6%). The yield of this crop was 50 mg. from 1 g. of calycanthine. The crude basic fraction may have contained a little of a rather less volatile base (homologue of quinoline?).

Actions of Calcium Oxide on Calycanthine.—The experimental conditions and optimal temperature (305°) were similar to those with soda-lime, but there was no pressure when the

tube was opened. The neutral fraction gave a distillate, b. p. 120—160°/15 mm., in a yield of 10% of the calycanthine used. This distillate consisted of indolic substances, of which only indole itself could be obtained pure. The mixture was partially separated at 15 mm. into fractions (a), b. p. 130—140° (Found: C, 80·7; H, 7·4; N, 10·7. Calc. for C_9H_9N: C, 82·4; H, 6·9; N, 10·6%. Found for the corresponding picrate, m. p. 158°: C, 49·0; H, 3·3; N, 15·2. Calc. for $C_9H_9N,C_6H_3O_7N_3$: C, 50·0; H, 3·3; N, 15·5%), and (b), b. p. 140—160° (Found: C, 80·5; H, 7·5; N, 9·9. Calc. for $C_{10}H_{11}N$: C, 82·7; H, 7·6; N, 9·7%). The carbon content is unaccountably low, as already referred to in other cases. (a) seems to be a methylindole, (b) a dimethyl- or an ethyl-indole.

On distillation at 15 mm. the basic fraction yielded crystals, b. p. 240—250°, mixed with a vitreous substance, b. p. 240—280°. From a solution of the distillate in acetone, the vitreous substance separated in long yellowish needles, m. p. 296°, and these, after repeated sublimation at atmospheric pressure over soda-lime, melted at 310° (Found: C, 83·3; H, 4·3. Calc. for $C_{16}H_{10}N_2$: C, 83·5; H, 4·3%). This was the purest specimen of calycanine we have obtained. The acetone filtrate from this substance was evaporated, and the residual oil dissolved in benzene. On concentration a second *base* crystallised in well-formed needles, m. p. 176°, and 183° after sublimation in a high vacuum (the m. p. depends greatly on the rate of heating and may be found as high as 190°). Yield, 15 mg. per g. of calycanthine (Found: C, 79·2; H, 5·5; N, 14·7; active H, 0·29. $C_{12}H_{10}N_2$ requires C, 79·1; H, 5·5; N, 15·4; 1 active H, 0·51%. The sample for the nitrogen determination had not been sublimed). The substance gave no coloration with *p*-dimethylaminobenzaldehyde or with glyoxylic acid; the pine wood test was inconclusive. The base was moderately soluble in water and showed an intense blue fluorescence in neutral or acid solution, indicating a methylcarboline related to harman. In some experiments this carboline was mixed not only with calycanine, but also with *N*-methyltryptamine, from which amine it could not be separated by the process outlined. The mixture of amine and carboline was in such cases dissolved in alcohol; addition of picric acid then caused the separation of the *methylcarboline picrate* in long needles, m. p. 252°, while the amine picrate remained in solution (Found: C, 52·8; H, 3·4; N, 17·1. $C_{12}H_{10}N_2,C_6H_3O_7N_3$ requires C, 52·6; H, 3·2; N, 17·0%).

When calycanthine was heated with quick-lime at 305° for 4 hours only, much less of the indoles and very little carboline were formed, but 30 mg. of *N*-methyltryptamine and 60 mg. of calycanine per g. of alkaloid. It would seem that the indoles are formed from the *N*-methyltryptamine by a secondary reaction. The shorter heating at 305° resulted also for the first time in an acid fraction (15 mg. of benzoic acid), which was probably decarboxylated in more prolonged experiments. Below 305° most of the alkaloid was recovered unchanged, along with only a little calycanine. At 260° a small quantity of a basic oil was obtained, b. p. 270°/15 mm. Its crystalline *picrate* melted at 194° (Found: C, 51·1; H, 4·8; N, 16·2. $C_{12}H_{16}N_2,C_6H_3O_7N_3$ requires C, 51·8; H, 4·6; N, 16·8%. $C_{12}H_{18}N_2,C_6H_3O_7N_3$ requires C, 51·5; H, 5·0; N, 16·7%). This unsatisfactory analysis suggests the picrate of a hydrogenated methylcarboline, which is perhaps dehydrogenated to the carboline at a higher temperature.

Action of Calcium Oxide on Benzoylcalycanthine.—When the benzoylated product from 1 g. of calycanthine was heated at 300° as above, the basic fraction obtained yielded 10 mg. of calycanine, and the neutral fraction an oil, b. p. 300°/1 mm., which crystallised from acetone–ether and then melted at 200°. It was identified as benzoyl-*N*-methyltryptamine. Unlike dry soda-lime, calcium oxide does not entirely remove the benzoyl group.

Action of Soda-lime on Calycanine.—The base (120 mg.) was mixed with soda-lime (2·5 g.) and heated in a sealed tube at 350° for 15 hours. There was no pressure and only a faint odour of indole; 90 mg. had sublimed unchanged out of the soda-lime. The basic fraction extracted from the soda-lime also consisted entirely of unchanged calycanine; the neutral fraction gave just enough of an indole to give the Ehrlich test; the acid fraction deposited from alcoholic solution a mixture of well-formed crystals, orange and yellow. It was separated mechanically under the microscope, 3 mg. of the orange *acid* being obtained, and even less of the yellow substance. The orange acid (III) melted at 355° (Found: C, 77·8; H, 4·0. $C_{16}H_9O_2N$ requires C, 77·7; H, 3·6. $C_{16}H_{11}O_2N$ requires C, 77·1; H, 4·4%). There was not enough for a nitrogen determination, but it seems evident that one of the original two nitrogen atoms has been lost.

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