C. THE CONSTITUTION OF GALEGINE.

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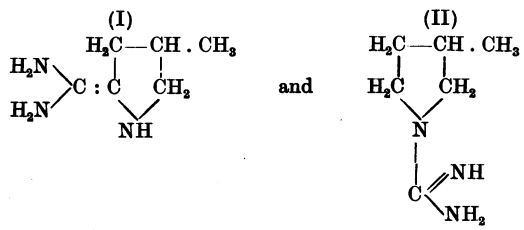
Tanret [1914] isolated from the seeds of *Galega officinalis* a base of the formula $C_6H_{13}N_3$, which is hydrolysed by baryta according to the equation:

$$C_6H_{13}N_3 + H_2O = CON_2H_4 + C_5H_{11}N$$

into urea and a volatile base; the latter Tanret (erroneously) regarded as 3-methylpyrrolidine, which led him to suggest two possible formulae for galegine:



Neither formula accounts for the properties of galegine, as given by Tanret. According to both, galegine would possess an asymmetric carbon atom; yet this base has no optical activity, nor could Tanret resolve it. Moreover, substances of either constitution would give monobenzoyl derivatives (or in the case of formula (I) possibly a tribenzoyl compound); yet Tanret obtained a *di*benzoyl derivative of galegine. These discrepancies induced us to re-investigate this question, particularly since the formation of urea by hydrolysis suggested that galegine is a guanidine derivative, and naturally occurring guanidine derivatives are rare. Tanret had indeed already considered its relationship to arginine (see note at the end of this paper).

We soon established the presence of a guanidine nucleus, because galegine gives Weyl's test and the diacetyl reaction [Harden and D. Norris, 1911] and is precipitated by silver nitrate in ammoniacal solution. The correct identification of the other fission product $C_5H_{11}N$ was a more lengthy process. Tanret had found that the boiling point of this base (105–108°) and the melting point of its platinichloride (194–196°) correspond closely to those recorded for 3-methylpyrrolidine (103–105° and 194–196° respectively); the agreement with the corresponding data for piperidine (B.P. 106°, M.P. of platinichloride 198–200°) is, however, almost as good. But when we had prepared the corresponding aurichloride and picrate, it was evident that the base was not identical with either 3-methylpyrrolidine or with piperidine. In order to choose between

the several other possible constitutions, a closer examination was necessary, which yielded the following results:

1. The volatile base gives Hofmann's carbylamine reaction.

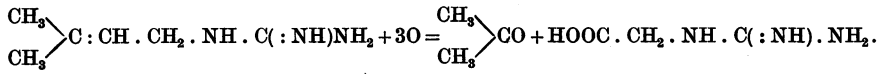
2. Treated according to Hinsberg, with toluenesulphonyl chloride it gives a toluenesulphonamide soluble in sodium hydroxide.

3. It decolorises potassium permanganate in dilute sulphuric acid solution [Willstätter, 1900].

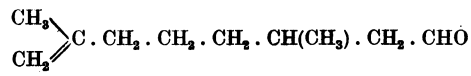
Observations 1 and 2 prove the base to be a primary one, observation 3 proves it to be unsaturated: it is therefore an amino-amylene.

In conformity with this view, galegine was found to take up exactly one molecule of hydrogen when reduced with palladium as catalyst, and the resulting dihydrogalegine yielded on distillation with lime *iso*-amylamine.

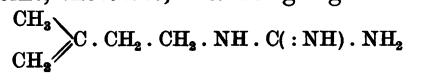
Now only the position of the double bond in galegine remained to be determined. This was done by oxidising galegine sulphate with barium permanganate, which yielded both acetone and glycocyamine (guanidino-acetic acid). The constitution was now almost established, for the oxidation may be expressed thus:



We say "almost established" for in the analogous case of citronellal, it was shown by Harries and Schauwecker [1901], after much controversy, that the constitution is



which does not prevent the formation of acetone [Tiemann and Schmidt, 1897]. It is a fine point, therefore, whether galegine has not the constitution



instead of that given in the above equation. It may perhaps be possible to settle this by a similar method to that employed by Harries and Schauwecker [1901] for citronellal.

Boiled with dilute sulphuric acid, galegine becomes stable to permanganate and takes up a molecule of water, forming hydroxydihydrogalegine; probably the hydroxy group is attached to the tertiary carbon atom.

Although the constitutions of galegine and its hydration product have still slight elements of uncertainty, such uncertainty does not arise in the case of dihydrogalegine; its constitution was, moreover, placed beyond doubt by its synthesis from *iso*-amylamine and cyanamide. The analogous synthesis of galegine itself would require the unsaturated amine $C_5H_{11}N$; very few bases of this type are known, for their synthesis presents considerable difficulty. It is indeed remarkable how few unsaturated simple bases occur in nature. Galegine is even more interesting in this respect than in the possession of a guanidine nucleus.

Methods of isolating galegine from the seeds.

The seeds were obtained and extracted for us by the British Drug Houses, Ltd. for whose help we are much indebted. From a portion of the extract galegine sulphate was first isolated by following Tanret's directions exactly, but we found his elimination of acetic acid by ether and removal of the sugar very tedious and afterwards greatly simplified the process. The extract (obtained after evaporating the 60 % alcohol used in extraction) contained much fat and was ground up with sand to a stiff paste and extracted several times with cold water. The combined decantations and final filtrate were treated successively with normal and basic lead acetate, the lead was removed as sulphate, the excess of sulphate ions quantitatively by baryta, and the filtrate was evaporated to a thin syrup. On adding 50 % sulphuric acid until acid to Congo the galegine sulphate ultimately crystallised in a yield of 0·5 % of the seeds, the same as that obtained by Tanret. After we had found that galegine is precipitated by silver nitrate and ammonia, we worked up the mother liquors by Kossel and Kutscher's method for the isolation of arginine, but only obtained a very small additional amount. Galegine, being unsaturated, is even more readily oxidised by silver in alkaline solution than creatinine [cf. Ewins, 1916]. In any case the solubility of galegine sulphate in cold water is so small, that but little is lost in the mother liquors. We also used an alternative method of isolation by precipitation with potassium bismuth iodide, regenerating with lead hydroxide. The resulting syrup would not at once yield the sulphate; it indeed readily gave the picrate, but as the regeneration of the latter is troublesome (see below) this alternative method is not recommended.

Colour reactions of galegine.

(*a*) Weyl's reaction. 2 cc. of a very dilute solution of sodium nitroprusside is made alkaline with 2 drops of 10 % sodium hydroxide and one drop of a 1 % solution of galegine sulphate is added; after standing for 15 minutes a red coloration had developed; limit 1 : 6000.

(*b*) Diacetyl reaction. One drop of diacetyl (prepared according to Diels and Stephan [1907]) is dissolved in 5 cc. of water and 2 drops of 10 % sodium hydroxide are added. On further adding one drop of a 0·5 % solution of galegine sulphate and warming slightly, the characteristic pink coloration of guanidine derivatives is produced. Limit 1 : 10,000. Under similar conditions we found the limit for arginine nitrate 1 : 5000.

Reduction of galegine; dihydrogalegine and its salts.

0·88 g. galegine sulphate (5 millimols of base) was dissolved in 25 cc. of water, 0·02 g. palladium chloride in a little very dilute hydrochloric acid was added, and the solution was shaken in a hydrogen atmosphere under a pressure of 2 atmospheres. The absorption was complete in three hours; no gum arabic was necessary.

Hydrogen absorbed: 115 cc. at 11° and 752 mm. = 109 cc. at N.T.P.
Hydrogen calculated for 5 millimols 112 cc. ,,

The catalyst, which had coagulated, was filtered off, and the solution was evaporated to a syrup. On dissolving this in boiling alcohol and cooling, dihydrogalegine sulphate crystallised in colourless prisms, M.P. 270° (galegine sulphate melts at 227°).

0·2033 gave 0·1336 $BaSO_4$ = 27·6 % H_2SO_4; $(C_6H_{15}N_3)_2H_2SO_4$ = 27·2 % H_2SO_4.

The sulphate is only slightly soluble in alcohol and water, yet more so than galegine sulphate. Weyl's and the diacetyl reaction are similar to those with galegine, but less sensitive. Permanganate and bromine water are not decolorised.

Dihydrogalegine nitrate is little soluble in dilute nitric acid but fairly readily in water and alcohol, and forms long needles from alcohol and ether, M.P. 75–76°.

Dihydrogalegine picrate is almost insoluble in cold water and crystallises from hot water in long narrow plates, M.P. 172°.

1 g. of the dry sulphate was mixed with 4 g. of quick lime and distilled. Besides ammoniacal vapours, it yielded (1) a colourless liquid, B.P. 89–95°; (2) a small fraction, B.P. 95–105° from which minute crystals separated, resembling cholesterol; (3) a yellow viscous mass, remaining in the flask, partly sublimed and partly distilled at 175°/0 mm., forming an oily liquid which solidified to a partially crystalline wax. This was insoluble in dilute acids, but soluble in concentrated hydrochloric acid, from which it was precipitated by ammonia. It is also soluble in glacial acetic acid and yields a picrate, after the solution has been diluted by water. A similar substance has been observed by Tanret on distilling galegine. We mention its production in the present case as evidence that its formation is independent of the unsaturated linking. The feeble base, which is formed perhaps from any alkylguanidine, requires further investigation.

The first fraction of the distillate was redistilled twice over solid potassium hydroxide and then over a mixture of sodium and potassium when it boiled pretty constantly at 95–96°. It had a strong ammoniacal odour, fumed in air and readily formed a carbonate, gave a white precipitate with Nessler's reagent, was stable to dilute acid permanganate and gave the carbylamine reaction.

The hydrochloride (long thin plates from acetone) M.P. 215° and the picrate M.P. 130–134° were found to be identical with the corresponding salts prepared from commercial *iso*-amylamine. A mixture of the hydrochlorides melted at 214°, of the picrates at 130–133°. *Iso*-amylamine picrate was prepared by adding an ethereal solution of picric acid to the amine, and recrystallised, on standing, from an ethereal solution containing a little acetone. On rapidly evaporating the ethereal solution, this picrate was obtained in fine needles. The amine from dihydrogalegine is therefore *iso*-amylamine.

Synthesis of dihydrogalegine (iso-*amylguanidine*).

Following the method used by Kossel [1910] for the synthesis of agmatine, we kept a concentrated aqueous solution of 1 g. cyanamide and 1·9 g. *iso*-amylamine for ten days at room temperature. Unchanged *iso*-amylamine was then boiled off and a cold saturated solution of picric acid added. A voluminous precipitate was immediately formed; recrystallised from boiling water, it formed long narrow plates, similar to those of dihydrogalegine picrate; yield 2·1 g. = 27% of the theory. On further recrystallisation the picrate melted at 171° (dihydrogalegine picrate from galegine 172°). A mixture of the two specimens melted at 171–173°. The regeneration of this picrate, like that of galegine, was difficult on account of its very slight solubility and the fact that guanidine derivatives are powerful bases. Boiling with 10 % sulphuric acid only decomposed an insignificant proportion. The method finally adopted was to dissolve in warm glacial acetic acid, dilute with ether, and extract many times with dilute sulphuric acid. After washing the aqueous solution with ether and removing the sulphate ions quantitatively with baryta, a syrup resulted on evaporation, which on addition of nitric acid yielded the nitrate. This after recrystallisation melted at 75–76°, and, mixed with the nitrate of the reduction product of galegine, at 74–76°.

This further proves the identity of dihydrogalegine with *iso*-amylguanidine.

Oxidation of galegine.

Kutscher [1901] obtained guanidinobutyric acid by oxidising arginine with barium permanganate in alkaline solution. His yield was poor for most of the arginine was oxidised to guanidine. Since we wished to oxidise galegine at the double bond, we considered it better to use only a small quantity of permanganate and to work in acid solution.

0·88 g. galegine sulphate (= 5 millimols of base) was dissolved in 60 cc. of 5 % sulphuric acid and 1·88 g. barium permanganate (= 5 millimols) was added. This could at most yield 5 atoms of oxygen. The purple colour at once disappeared but, although the mixture was boiled, manganese dioxide remained undissolved, and only 3 atoms of oxygen were used up. The mixture of barium sulphate and manganese dioxide was filtered whilst hot, and the excess of sulphuric acid removed from the filtrate with barium carbonate. The solution was then concentrated to half its bulk by distillation, and the distillate kept to demonstrate the formation of acetone (see below). The solution was then evaporated to dryness on the water-bath, when a considerable amount of a solid remained which recrystallised from boiling water in acicular plates. On slow cooling these formed characteristic sheaves; they melted at 270–280°. The substance was little soluble in cold water but readily in both acids and alkalis. It gave the diacetyl reaction, but Weyl's reaction only very faintly and Jaffé's reaction not at all.

Micro-Kjeldahl: 6·21 mg. gave 2·25 mg. N = 36·2 %
4·50 mg. ,, 1·64 mg. N = 36·4
Calculated for glycocyamine, $C_3H_7O_2N_3$ N = 35·9

For purposes of direct comparison we prepared some *glycocyamine* by heating chloroacetic acid with 5 molecular proportions of free guanidine in concentrated aqueous solution to 60° for two hours [Ramsay, 1908]. It was found to be identical with the oxidation product of galegine.

The hydrochlorides were prepared by evaporating a solution of the substance in hydrochloric acid, and formed colourless plates. M.P. (from galegine) 190°; synthetic 191°; mixture 189°.

The picrates formed long needles from hot water. M.P. (from galegine) 201–203°; synthetic 202°; mixture 201°.

As a further confirmation, a little of the oxidation product was heated with concentrated hydrochloric acid to 140° for two hours, so as to convert it into the anhydride, glycoamidine; the residue remaining on evaporation gave a deep blood-red colour with picric acid and sodium hydroxide (Jaffé's reaction).

Acetone was identified as the other oxidation product in the distillate obtained after removal of the sulphuric acid (above). It gave Legal's test with sodium nitroprusside and at once yielded a precipitate with *p*-nitrophenylhydrazine in 50 % acetic acid. The hydrazone crystallised from dilute alcohol in fine yellow needles, M.P. 148–149°. The melting point of the *p*-nitrophenylhydrazone of acetone is given as 148–148·5°. The yield was 0·49 g. or 50% of the theory.

The unsaturated amine $C_5H_{11}N$ *from galegine.*

As this amine was unknown, we had no material for direct comparison, and on account of the small amount available, we have not yet examined it very closely. We found it most convenient to prepare it directly from galegine sulphate by distillation with lime, as described above for dihydrogalegine; distilled over potassium hydroxide the amine boiled at 105–108°, as in Tanret's experiments; distillation over potassium did not, however, give a sharper boiling point, as it did in the case of *iso*-amylamine. This is evidently connected with unsaturation: potassium partially polymerised the product.

The amine is a primary base (carbylamine reaction; toluenesulphonamide soluble in alkali). It is unsaturated, at once decolorising permanganate and bromine water. It has a pungent, piperidine-like odour.

A rough determination of the density on the small amount of approximately pure distillate gave

$$D\,\frac{18°}{18°} = 0{\cdot}779.$$

Although this determination is not accurate, it suffices to establish the fact that the amine is unsaturated and not cyclic. The value $D^{18°}_{18°} = 0{\cdot}779$ is much (about 0·06) lower than that of cyclic amines of the formula $C_5H_{11}N$. Thus piperidine has $D^{18·6°} = 0{\cdot}8619$ and 3-methylpyrrolidine has $D^{0°}_{4°} = 0{\cdot}8654$. On the other hand the density found is slightly higher than that of the corresponding saturated *iso*-amylamine, from which it differs by the same amount as the densities of an amino-hexylene and the corresponding saturated hexy-

lamine. From the densities given in Beilstein (4th ed.) we calculate for *iso*-amylamine $D^{18°}_{18°} = 0{\cdot}753$, whence increment for a double bond would be 0·026. The density of $CH_2 : CH . CH_2 . CH_2 . CH(CH_3) . NH_2$ is given as $D^{15°}$ 0·779, of $CH_3 . CH_2 . CH_2 . CH_2 . CH(CH_3) . NH_2$ as $D^{20°}_{0°} = 0{\cdot}753$, whence a slightly smaller increment for the double bond may be deduced, corresponding to the higher position in the homologous series. For the much lower members of the series, allylamine ($D^{18°} = 0{\cdot}769$ approx.) and propylamine ($D^{18°} = 0{\cdot}720$ approx.) the increment is about 0·049.

In order to obtain the hydrochloride, a solution free from excess acid must be evaporated (alkaline to methyl red, but not to phenolphthalein). Excess of hydrochloric acid fixes itself on the double bond. The hydrochloride is somewhat hygroscopic, and very soluble. It was not analysed, but the following salts were prepared:

Platinichloride, from the solution of the amine in excess of hydrochloric acid, by adding aqueous platinic chloride solution. The yellow precipitate which separates at once, is crystallised from hot water; M.P. 194–197°.

41·9 mg. gave 14·13 mg. Pt	Pt = 33·7 %
Calculated for $(C_5H_{11}N)_2 . H_2PtCl_6$	Pt = 33·6 %

The same salt was obtained from the amine prepared according to Tanret, by hydrolysis of galegine by baryta at 100° and extraction with ether.

Aurichloride, from concentrated aqueous solutions of the hydrochloride and auric chloride. Rhomb-shaped plates from warm water, M.P. 57°. After drying *in vacuo* over sulphuric acid it sintered at 75° and melted at 81°. It was then analysed.

34·28 mg. gave 16·05 mg. Au	Au = 46·8 %
Calculated for $C_5H_{11}N . HAuCl_4$	Au = 46·35 %

Picrate. Ethereal solutions of the amine and of picric acid are mixed. It is moderately soluble in hot water, from which it crystallises in long narrow plates, M.P. 138·5–139·5°.

Action of hydrobromic and hydrochloric acids on the amine.

In an attempt to prepare the hydrobromide, the amine was evaporated to dryness with excess of hydrobromic acid. The residue, after drying over potassium hydroxide, crystallised from acetone, after adding ether, in fine colourless needles. The salt was not hygroscopic, but unstable to air and light and decomposed on rapid heating to 110°. It was therefore dried *in vacuo* at 78° over phosphorus pentoxide, when it melted at 206°.

Micro-Kjeldahl: 27·15 mg. gave 1·59 mg. N	N = 5·86 %
Calculated for $C_5H_{12}N . Br . HBr$	N = 5·67 %

Bromine estimation by direct precipitation with $AgNO_3$

0·1522 gave 0·2287 AgBr	Br = 63·9 %
Calculated for $C_5H_{12}N . Br . HBr$ for both bromine atoms	Br = 64·8 %

The salt is evidently the hydrobromide of a bromo-*iso*-amylamine which readily loses two molecules of hydrobromic acid. Whether the original un-

saturated amino-amylene is thereby reformed, or whether a new cyclic base results, has not yet been determined. In any case this does not appear to be methylpyrrolidine, since the bromo-*iso*-amylamine is not stable to permanganate in sulphuric acid solution.

Similarly chloro-*iso*-amylamine is obtainable by evaporating amino-amylene with hydrochloric acid. The hydrochloride of this chloro-amine is hygroscopic. It was converted into *the platinichloride* $(C_5H_{12}NCl)_2 . H_2PtCl_6$. This is much more soluble than the platinichloride of the unsaturated base, and is decomposed on boiling its aqueous solution. From dilute alcohol it formed iridescent six-sided plates, quite different in shape from the corresponding unsaturated salt, but having the same melting point (193–195°).

This salt decomposes at 110° and was therefore dried *in vacuo*.

35·13 mg. gave 10·88 mg. Pt	Pt = 31·0 %
23·93 mg. ,, 7·5 mg. Pt	Pt = 30·9 %
Calculated for $(C_5H_{12}NCl)_2 . H_2PtCl_6$	Pt = 29·9 %

Evidently this salt had already lost some hydrochloric acid, although the platinum content was still much below that of the unsaturated salt (33·6 %).

The aurichloride crystallises from warm water in plates, sintering at 90° and decomposing at 99–101°. It is also unstable, but was obtained practically pure by drying over sulphuric acid.

20·7 mg. gave 8·76 mg. Au	Au = 42·3 %
Calculated for $C_5H_{12}NCl . HAuCl_4$	Au = 42·7 %

The picrate formed by adding picric acid to the hydrochloride is very much more soluble than the unsaturated salt in water, alcohol or acetone. Washed free from picric acid with ether, it melted at the same temperature as the unsaturated picrate (137–138°) but contained chlorine.

Hydroxydihydrogalegine.

When converting a quantity of (the very slightly soluble) galegine picrate into the sulphate, the former was boiled for some time with dilute sulphuric acid, in order to dissolve it. The resulting sulphate was, however, very soluble in water, in contradistinction to galegine sulphate. It crystallised from methyl alcohol in fern-like crystals melting at 205–206°. (Galegine sulphate M.P. 227°; the mixture melted at 200°.)

The new sulphate was quite stable to potassium permanganate and to bromine water; it did not take up hydrogen in the presence of palladium, but it still gave Weyl's and the diacetyl reaction. The air-dry substance contains one molecule of water of crystallisation.

Dried at 120–130° it lost 4·1 %.

$(C_6H_{15}ON_3)_2 . H_2SO_4 . H_2O$ requires H_2O = 4·4 %.

The anhydrous substance was analysed.

0·2300 gave 0·3091 CO_2 and 0·1696 H_2O	C = 36·65; H = 8·2.
8·69 mg. (micro-Kjeldahl) gave 1·89 mg. N	N = 21·7.
0·2147 gave 0·1300 $BaSO_4$	H_2SO_4 = 25·4.

Calculated for $(C_6H_{15}ON_3)_2 . H_2SO_4$; C = 37·1, H = 8·0, N = 21·6, H_2SO_4 = 25·3.

On boiling with dilute sulphuric acid galegine therefore takes up a molecule of water, to form hydroxydihydrogalegine of the probable constitution:

$$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!C(OH).CH_2.CH_2.NH.C(:NH).NH_2$$

The transformation can be conveniently followed by potassium permanganate. Boiling with 25 % sulphuric acid renders it complete in five minutes; with 5 % sulphuric acid about 45 minutes are required.

Hydroxydihydrogalegine picrate separates from a concentrated hot aqueous solution in rhomb-shaped crystals, M.P. 153–154°, much more soluble than galegine picrate.

In order to regenerate galegine from the picrate it is therefore necessary to avoid boiling with mineral acids. It can be done by dissolving in alcohol, diluting with much ether and extracting with successive small quantities of 50 % sulphuric acid. The aqueous solution is washed with ether and the sulphuric acid is removed quantitatively by baryta; the yield is unsatisfactory.

Hydroxydihydrogalegine yields on hydrolysis a hydroxy-*iso*-amylamine, which can also be obtained by boiling the unsaturated amine with sulphuric acid, but has not been examined further as yet. Its synthesis is being attempted, with a view to that of galegine.

This investigation was rendered possible by a grant to one of us (F.D.W.) from the Department of Scientific and Industrial Research. The cost of material and its extraction was met by a grant of the Moray Research Fund of this University. For both grants we wish to express our gratitude.

[*Note added November 22nd.*] This paper was submitted in MS. to M. Georges Tanret, who declared himself in agreement with our conclusions and sent us a copy of a thesis of the University of Paris, 1917, entitled *Recherches chimiques et physiologiques sur la graine de Galega*, in which (p. 34) he had considered in a footnote the possibility that galegine is a guanidyl derivative of an unsaturated *iso*-amylamine; he considered that on hydrolysis with baryta the amine is isomerised to 3-methylpyrrolidine. As we have shown, this latter supposition is incorrect.

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