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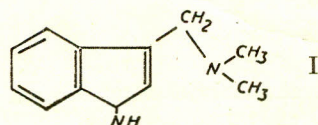
April 1958

SOME REACTIONS OF GRAMINE

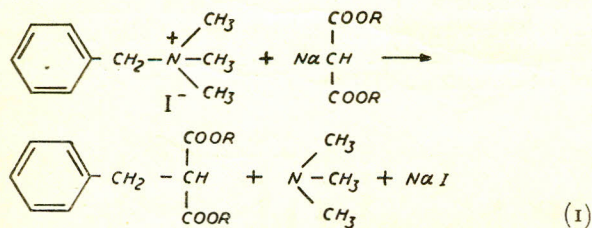
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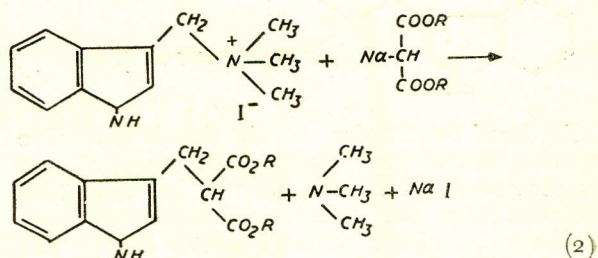
Gramine (I) was first isolated out of chlorophyll deficient mutants of barley by v. Euler and Hellstroem¹ in 1932 and was soon found to be identical with the alkaloid donaxine out of *Arundo Donax* (*Gramineae*).² Kuhn and Stein³ found a synthesis using the Mannich reaction which, applied on indole with formaldehyde and dimethylamine in acetic acid solution gives quantitatively pure gramine.



It gained considerable importance when Snyder⁴ in 1944 used it for an excellent synthesis of tryptophane. This achievement was based on studies of the reaction of quaternary ammonium salts containing the benzyl moiety. According to equation 1 they were found to act as alkylating agents yielding benzyl malonate (65%).



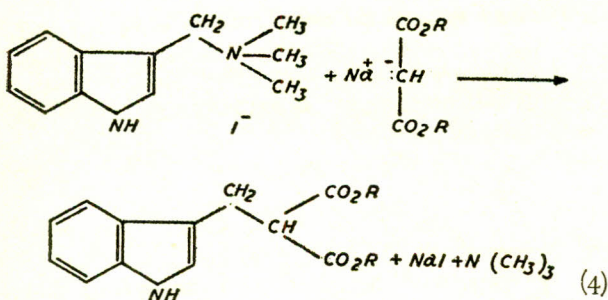
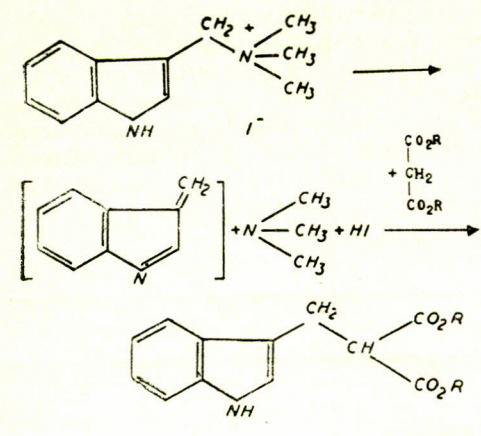
By using graminemethyl iodide, Snyder found that this salt behaves in the same manner furnishing skatyl malonate with even better yields (85%) according to equation (2).



Thus the way for the tryptophane synthesis was prescribed. Starting with acetyl aminomalonnate, he got skatyl-acetyl-aminomalonnate, which on saponification gave tryptophane in excellent yields.

Two possible mechanisms for this reaction have been put forward by Snyder et al.⁵ The first consists of an elimination of trimethylamine hydroiodide and a subsequent Michael type of addition according to that of Mannich⁶ for alkylations with salts from ketonic Mannich bases (cf. equation 3).

In the second mechanism a molecule of trimethylamine is displaced from the quaternary gramine-ion by the anion of the substance being alkylated (cf. equation 4).

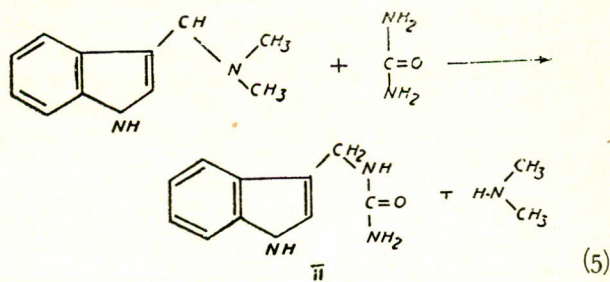


Furthermore Snyder et al. found that free gramine reacts very slowly with malonic ester but traces of alkali catalyse this process. This indicates that the reaction depends not only on the polarisation of the C-N bond in benzyl- and skatyl-amines but also on the reactivity of the hydrogen atom of the compound being alkylated. It was therefore thought worthwhile to investigate the reaction of gramine with substances containing hydrogen of various reactivity both with and without alkali as a catalyst.

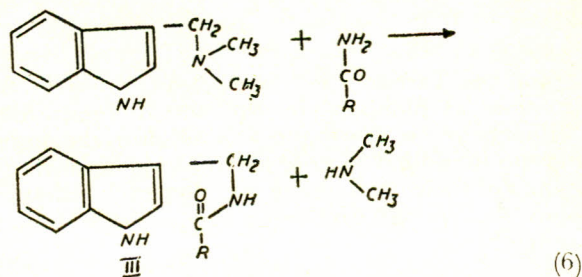
The first group of substances were amides beginning with urea. By simply melting gramine in an excess of urea which served as a solvent, and keeping the temperature below 150°C. where biuret formation takes place, 46% of skatylurea (II) were formed (cf. equation 5).

By adding a trace of alkali the yield goes up to 70%. In addition to microanalysis for all elements, the constitution of skatylurea has been confirmed by preparing the nitroso compound thus showing that skatylurea behaves like other monosubstituted ureas.

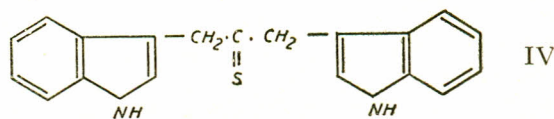
Next, acetamide, phenylacetamide, benzamide, urethane, succinamide and adipicamide were



brought into reaction with gramine. Though dimethylamine was always evolved, the mixtures soon turned to dark masses out of which no crystalline material could be obtained. With traces of alkali the same reactions had already been carried out by Hellmann,⁸ who found that condensation takes place with yields not more than 20%. So the idea to get compounds of structure III through this method had to be abandoned.

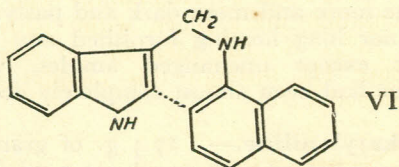
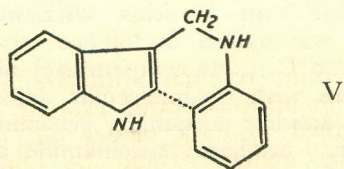


The exceptional behaviour of urea is the more remarkable as thiourea reacts in a completely different manner. Melted with gramine without alkali, sulphur containing gaseous decomposition products are evolved even at low temperature, the whole mass soon darkens and no crystalline substance could be isolated. Because urea and gramine form also skatylurea in alcoholic solution, the same conditions were used with thiourea. Soon a colourless crystalline substance came out of the clear solution which turned out to be something completely different from skatyl-thiourea.⁷ Having only two nitrogens instead of three, the compound analyses for a substance to which the tentative formula of a *sym.*ββ- diindolyl-thioacetone (IV) was given.

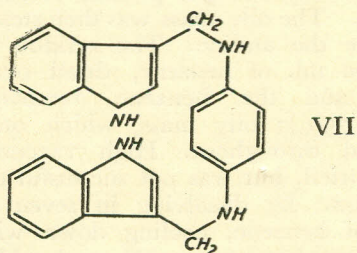


⁷ This reference was so for not available to authors.

The peculiar behaviour of urea may be explained by the fact that urea has a remarkable basic character represented by its nitrate formation, while the amides used have more or less acidic properties, forming sodium salts. Consequently aniline, α -, and β -naphthylamine were caused to react with gramine and yielded without alkali practically quantitatively the corresponding structures V and VI, respectively. Dehydrogenation



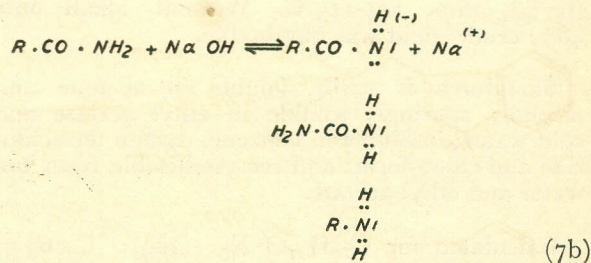
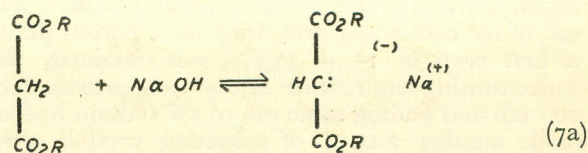
(not yet carried out), may lead to 5-carbolin systems as indicated by the dotted lines. Substituents in the *o*-, *m*-, and *p*-position to the amino-group in aniline seem to create steric hindrance in some of these compounds, e.g. *p*-phenylenediamine furnishes quantitatively the compound VII, while



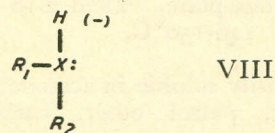
o-phenylenediamine yielded only something like 4% of the *o*-isomer. The chloroanilines as well as the anisidines react in the expected manner, but *m*-nitroaniline does not react at all.

Summarising these findings, we have on the one side malonic ester and the amides as non-reacting substances; and on the other, the same substances with alkali as a catalyst and also ammonia derivatives of sufficiently high basicity, like urea, piperidine, aniline and its substitution products as reacting agents. These findings can be explained by formulating the electronic state of the reacting components. It turns out that they all have one thing in common, namely, they can be considered to be krypto-anions⁸ or to become

so by mesomerism under the catalytic effect of the alkali. While malonic ester and the amides are scarcely ionised, the catalytic effect of alkali favours a mesomeric state according to equation (7a) and (7b) where malonate and amide krypto-anions are formed. While any substance carrying an amino group, which is able to combine with a proton to form an ammonium salt, can be regarded to be already a krypto-anion.



On this basis the reactions of benzyl- or skatyl-moiety containing quaternary ammonium salts with malonate, of free gramine with malonic ester and amides in the presence of alkali as a catalyst, and finally of free gramine with urea and amines of sufficient basicity appear to be of one and the same mechanism, namely, a replacement of a volatile krypto-anion (here dimethylamine) by another one of the general type VIII, where X can be



carbon as well as nitrogen. The extent to which this hypothesis can be generalised is subject to further investigation.

Experimental

Skatylurea.—80 g. of urea (1.3 moles) was carefully melted over a free flame and 20.2 g. of diethylskatylamine* (1/10 mole) along with

* As dimethylamine was out of stock diethylamine had to be used to prepare diethylskatylamine in Kuhn and Stein's synthesis. The m.p. 160°C. for diethylskatylamine given in the literature must be a misprint. Crystallised out of acetone, it melts at 102-

0.9 g. of powdered sodium hydroxide added. A vigorous evolution of diethylamine takes place. After about 1/2 hr. shaking and constant heating upto not more than 130°C.—because biuret-formation starts at 150°C.—all diethylskatylamine had gone in solution. The clear and slightly coloured melt was heated for an additional 2 hrs. in nitrogen atmosphere, after which time the gas evolution practically ceased. Poured in 200 ml. water the melt starts crystallising at once. Left standing overnight, filtered under suction, washed with a few ml. of ice cold water and dried on a porous plate, a first crop of 15 g. (79%) was obtained. By concentrating the mother liquor to approximately 100 ml. and adding some ml. of 2N sodium hydroxide another 2.19 g. of colourless crystals were obtained. Crude yield 17.19 g. (90.6%), m.p. 136-139°C. Recrystallised from water 13.04 g. (69%), m.p. 144-145°C. Without alkali only 46% crude yield was obtainable.

Skatylurea is easily soluble in acetone and alcohol; sparingly soluble in ethyl acetate and cold water; insoluble in benzene, carbon tetrachloride and chloroform; and recrystallisable from hot water and ethyl acetate.

Calculated for $C_{20}H_{11}O N_3$ (189): C, 63.5; H, 5.8; O, 8.4; N, 22.2. Found: C, 63.5; H, 5.8; O, 8.4; N, 22.3.

Nitrososkatylurea.— 1.89 g. of skatylurea (1/100 mole) and 2.5 g. of sodium nitrite were dissolved in 10 ml. of water by heating. After cooling down with ice to approximately 5°C., 1 ml. of glacial acetic acid was added. Soon the yellow needles of the nitroso compound separated. After 3/4 hrs. standing in ice the material was filtered under suction, washed with cold water and dried on a porous plate. Yield 2.16 g. (99%), decomposes at 146-150°C.

It is easily soluble in acetone; sparingly soluble in ether, petrol ether, carbon tetrachloride, chloroform and benzene; and insoluble in ethyl acetate, alcohol and dioxane. Being unstable to heating in solution, it was recrystallised by dissolving in cold acetone and precipitation with petrol ether, m.p. 150°C. dec.

Calculated for $C_{10}H_{10}O_2N_4$ (218): C, 55.0; H, 4.6; O, 14.7; N, 25.7. Found: C, 55.2; H, 4.5; O, 14.5; N, 25.5.

Gramine and Thiourea.— 1.74 g. of gramine (1/100 mole) and 0.76 g. (1/100 mole) plus 0.04 g. excess of thiourea were refluxed together with

5 ml. of alcohol in a glycerine bath heated up to 150°C. (bath temperature) for 10 hrs. On cooling, a white crystalline substance separated. Yield 1.65 g., m.p. 150-152°C. Recrystallised from alcohol, m.p. 154°C.

Calculated for $C_{19}H_{18}N S_2$ (306): C, 75.1; H, 5.3; N, 9.21; S, 10.5. Found: C, 74.6; H, 5.9; N, 9.3; S, 10.6.

Gramine and Amides without Alkali.— Gramine was heated in toluene on a glycerine bath at 140°C. (bath temperature) in a nitrogen atmosphere with equimolecular amounts of the following amides: acetamide, benzamide, phenylacetamide, urethane, succinamide and adipicamide. In all cases, a very slow evolution of dimethylamine took place, while the reaction mass became more and more dark and pasty. Neither short nor long heating furnished any crystalline matter except unchanged amides, while the gramine had been almost completely decomposed.

N-Skatylaniline.— 17.4 g. of gramine (1/10 mole) was heated with 40 ml. (41.3 g.) (1/10 mole plus an excess of 31.8 g.) of freshly distilled aniline in a glycerine bath under nitrogen atmosphere. Evolution of dimethylamine started at 100°C. and became vigorous at 135°C. bath temperature. Gramine slowly went into solution. The heating at 125°C. (bath temperature) was continued until the dimethylamine was no longer traceable (14 hrs.); only a slight pinkish colouration was produced. The oily mass was then steam distilled to remove the aniline. The residue was taken up in 100 ml. of benzene, dried over sodium sulphate and the benzene removed. Yield, 16.7 g. (75%); oily mass, which on standing crystallised throughout. High vacuum distillation was tried, but was not successful due to decomposition. By dissolving in seven times the volume of benzene, cooling down with ice to temperatures between 0-5°C. and adding dropwise cooled petrol ether until turbidity sets in, colourless crystals melting at 83-85°C. can be obtained. On exposure to air the substance becomes reddish. It is difficult to crystallise.

N-Skatylaniline is easily soluble in benzene, alcohol, ethyl acetate, acetone and acetic acid; and is insoluble in petrol ether and water.

Calculated for $C_{15}H_{14}N_2$ (222): C, 81.08; H, 6.30; N, 12.61. Found: C, 81.95; H, 6.36; N, 11.91.

N-Skatyl- α -naphthylamine.— 1.74 g. of gramine (1/100 mole) was heated together with 1.5 g. (1/100 mole plus an excess of 0.07 g.) of α -naphthylamine under nitrogen atmosphere

103°C. Our preparation has been confirmed by analysis.

Calculated for $C_{13}H_{18}N_2$ (202): C, 77.2; H, 8.9; N, 13.9. Found: C, 76.96; H, 9.0; N, 14.3.

in a 50 ml. round bottom flask in a glycerine bath. The mixture melted at 110-120°C. (bath temperature) and evolution of dimethylamine gas started. It was heated at this temperature until dimethylamine was no longer traceable, (30 hrs.) and kept over-night in a nitrogen atmosphere. After that time the dark brown oily mass had crystallised throughout. Yield 2.69 g.; easily soluble in benzene, ethyl alcohol, ethyl acetate, acetone and acetic acid; and insoluble in petrol ether and water. Recrystallisable out of benzene - petrol ether.

The whole mass was dissolved in 20 ml. of benzene, cooled, and ice-cold petrol ether was added dropwise until turbidity appeared (11 ml.), and was left in the refrigerator over-night. The first crop of crystals yielded 0.75 g., m.p. 148-150°C. To the mother liquor another 4 ml. of ice-cold petrol ether were added dropwise until the liquid became turbid and the mixture was kept standing over-night in the refrigerator. Another fraction of 0.89 g. could be obtained of the same m.p. (148-150°C.). Total yield 1.64 g. (60%). This pure product could be recrystallised out of benzene alone. The m.p. remained unchanged.

Calculated for $C_{19}H_{16}N_2$ (272): C, 83.82; H, 5.88; N, 10.29. Found: C, 83.72; H, 5.80; N, 10.76.

N-Skatyl- β -naphthylamine.— 1.74 g. of gramine (1/100 mole) was heated together with 1.5 g. (1/100 mole plus an excess of 0.07 g.) of β -naphthylamine in nitrogen atmosphere in a 50 ml. round bottom flask in a glycerine bath. The mixture melted at 120-130°C. (bath temperature) and the evolution of dimethylamine gas started. The gas evolution stopped practically after 27 hrs. heating, during which time small amounts of the β -naphthylamine sublimated out of the reaction mass. Solubilities and recrystallisation was the same as with the α -isomer. Total yield 1.50 g., m.p. 185-187°C. This product could also be recrystallised out of benzene alone for analysis.

Calculated for $C_{19}H_{16}N_2$ (272): C, 83.82; H, 5.88; N, 10.29. Found: C, 84.64; H, 6.28; N, 9.57.

N-Skatyl-*o*-chloroaniline.— This substance was prepared in the same way out of gramine and *o*-chloroaniline. Crude material out of benzene-petrol ether: 42%. It was recrystallised a second time out of benzene-petrol ether and gave a m.p. of 70-72°C.

Calculated for $C_{18}H_{13}N_2Cl$ (256.5): C, 70.18;

H, 5.06; N, 10.91; Cl, 13.84. Found: C, 70.43; H, 5.51; N, 10.54; Cl, 13.34.

N-Skatyl-*p*-chloroaniline.— Gramine and *p*-chloroaniline yielded 36% of the condensation product from benzene-petrol ether, m.p. 98-100°C.

Calculated for $C_{18}H_{13}N_2Cl$ (256.5): C, 70.18; H, 5.06; N, 10.91; Cl, 13.84. Found: C, 71.39; H, 5.28; N, 10.49; Cl, 12.88.

N-Skatyl-*m*-chloroaniline.— The reaction between gramine and *m*-chloroaniline was carried out in the same manner as described before. By recrystallisation from benzene and petrol ether only an oily substance could be obtained in spite of repeating the procedure seven times with long standing in the refrigerator. Due to the sensitivity against acids—all these skatyl derivatives become red at once—salt formation could not be used for further purification.

N-Skatyl-*m*-nitroaniline.— From the reaction between gramine and *m*-nitroaniline too, dimethylamine was developed, but only dark reddish decomposition products could be observed to have been formed.

N,N'-Diskatyl-*p*-phenylenediamine.— 3.44 g. of gramine (1/50 mole) and 1.08 g. of *p*-phenylenediamine (1/100 mole) had been well mixed in a mortar and heated in nitrogen atmosphere in a glycerine bath. The mixture melted at 130°C. and the development of dimethylamine followed almost immediately. After 5 hrs. heating at 135°C. (bath temperature) 80% of the theoretical amount of dimethylamine had been evolved, as determined by repeated weighing of the reaction flask. The gas evolution was then very slow and the experiment was stopped. The crude mass melted at 170-175°C. By treating with a few drops of benzene, the melt recrystallises and 2.7 g. (73.7%), m.p. 174-176°C., could be obtained. After recrystallisation twice from ethyl acetate, the melting point was constant at 183-185°C. The substance is easily soluble in acetic anhydride (acetylation), sparingly soluble in petrol ether, ethyl acetate, acetone, ethyl alcohol; insoluble in benzene; and recrystallisable from ethyl acetate.

Calculated for $C_{24}H_{22}N_4$ (366): C, 78.68; H, 6.01; N, 15.3. Found: C, 78.6; H, 5.99; N, 15.47.

N,N'-Diacetyl- N,N'-diskatyl-*p*-phenylene-diamine.— 1.5 g. of N,N'-diskatyl-*p*-phenylene-diamine was spread in a thin layer in a petri dish and just enough acetic anhydride was added to moisten the substance. With development of

heat the substance goes in solution, and almost immediately the acetylated compound separated in well shaped prisms so that the whole mass became solid. After some standing and cooling, it was filtered under suction and washed with a few ml. of cooled anhydride, yield 1.5 g. (76.6%).

The acetyl compound is sparingly soluble in glacial acetic acid, dioxane, ethyl alcohol and benzene; and recrystallisable by dissolving in glacial acetic acid with heating and after some concentration precipitating with ethyl acetate, m.p. 250-252°C.

Calculated for $C_{28}H_{26}O_2N_4$ (450): C, 74.66; H, 5.77; N, 12.44; O, 7.11. Found: C, 73.94; H, 5.76; N, 11.65; O, 8.46.

N,N' - Diskatyl - o - phenylenediamine.—

This reaction was carried out in the same way as with the *p*-phenylenediamine, but very little crystalline substance could be isolated from the oily reaction product. This substance (in about 5% yield) melted at 185-195°C., and behaved in the same manner against acetic anhydride as the para-isomer furnishing 4% of theoretical yield of acetylation product, m.p. 221-223°C. from ethyl acetate.

Calculated for diacetyl product $C_{28}H_{26}O_4N_4$ (450): C, 74.66; H, 5.77; N, 12.44; O, 7.11. Calculated for tetraacetyl product $C_{32}H_{30}O_6N_4$ (534): C, 71.9; H, 5.4; N, 10.4; O, 11.9. Found: C, 71.34; H, 6.15; N, 12.63; O, 9.71.

N-Skatyl -*p*- anisidine.— 3.48 g. of gramine (1/50 mole) and 2.4 g. of *p*-anisidine (1/50 mole) were well mixed in a mortar and heated on a water

bath. Dimethylamine developed as soon as the mixture melted and continued to come out during 17 hrs. After that the mass crystallised on cooling. By making a paste with ethyl alcohol, the oily impurities were removed. A colourless material 2.5 g. (50%) was obtained, m.p. 94-99°C.

It is easily soluble in acetone; sparingly in ether, benzene, ethyl acetate, ethyl alcohol and chloroform; insoluble in petrol ether; and recrystallisable from ethyl alcohol, m.p. 188-190°C.

Microanalyses were carried out by A. Bernhardt, Max Plank Institut, für Kohleforschung, 22 a Mulheim (Ruhr), W. Germany. Substances were dried over phosphorus pentoxide at 50°C. in a high vacuum before analysis.

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