EXPERIMENTS ON VITEXIN AND VERATRINE.

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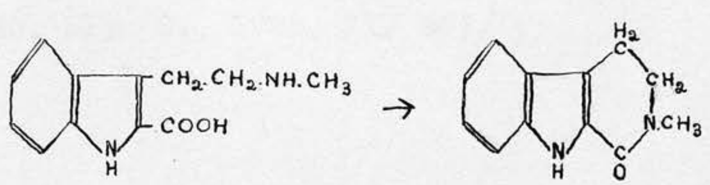
Contents

NOTE ON ABRININE.

In the first place work was carried out on the constitution of abrinine. (Abrinine is identical with the abrine of N. Ghatak et al and T. Hoshino, who overlooked the fact that the name abrine was reserved for a toxin from Abrus precatorius (cf. K. Braun, B., 1903, 36, 3003) which is mentioned also in textbooks (cf. K.O. Oppenheimer, Organische Chemie, 2nd edition, 1928, p. 452)). However, while this investigation was in progress, a paper appeared by T. Hoshino (Ann., 1935, 520, 31) which seemed to settle the constitution of this alkaloid. Further work on this subject was therefore discontinued, but a brief account will be given of the results obtained before the Japanese paper appeared. Abrinine was isolated by N. Ghatak and R. Kaul (J. Indian Chem. Soc., 1932, 9, 383) from the seeds of Abrus precatorius. Ghatak (Bull. Acad. Sci. Allahabad, 1934, 3, 295; C., 1935, I, 576) assigned to it the formula $C_{12}H_{14}O_2N_2$ and found an alcoholic hydroxyl and a secondary nitrogen. In the course of the present investigation it was found that abrinine colours pinewood dipped into concentrated hydrochloric acid red and gives a blue coloration with/

with a 2% solution of paradimethylaminobenzaldehyde in 20% hydrochloric acid (Ehrlich reagent), this reaction indicating the presence of the indole group. On heating with lime in vacuo at 300° indole and skatole were obtained. It was found that abrinine contains an N-methyl group. It contains no hydroxyl; the oxygen atoms are present in a carboxylic group, which on heating in any solvent above 175° is readily split off. With glyoxilic reagent and concentrated sulphuric acid, the violet ring indicating a free 2 position in the indole nucleus (Adamkievič reaction) appeared only on heating. This observation was interpreted as indicating that in abrinine the 2 position is occupied, but that after a short heating it becomes free and the violet colour is then produced. It was therefore conjectured that abrinine is a derivative of N-methyl tryptamine with a carboxylic group in the 2 position. A compound of this structure might be expected to lose water readily forming a harmane ring



which/

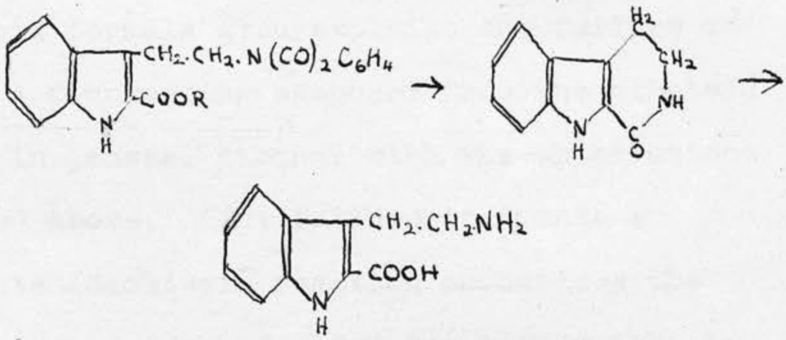
which would probably be fluorescent. However, attempts to isolate a fluorescent compound were not successful. Indole-3-ethylamino-2-carboxylic acid was prepared in the following way:

Phthalimido-bromopropane (cf. R.H.F. Manske and H.R. Ing, J.C.S., 1926, 2348) was condensed with ethyl acetoacetate (cf. R.H.F. Manske, W.H. Perkin, Jr. and R. Robinson, J.C.S., 1927, 8), the product condensed with diazotised aniline yielding a phenylhydrazone which on boiling with alcoholic hydrochloric acid closed to an indole ring (cf. S. Keimatsu, S. Sugasava and G. Kasuya, J. Pharm. Soc., Japan, 1928, <u>48</u>, 105; C., 1928, II, 1882). The phthalic acid group was removed by heating with 50% aqueous hydrazine hydrate followed by hydrolysis with 10% hydrochloric acid (cf. S. Keimatsu <u>et al</u>, loc. cit.). The amino acid thus obtained lost one mol of water closing to a harmane ring which was reopened by hydrolysis with strong aqueous potassium hydroxide (cf. Asahina <u>et al</u>, J. Pharm. Soc. 1928, 48, 51; C., 1928, II, 58).

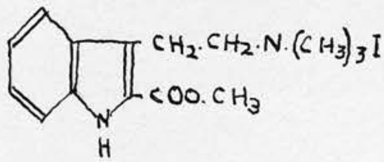
Br/

$BrCH_2CH_2CH_2N(CO)_2C_6H_4 \rightarrow CH_3COCH(COOH).CH_2CH_2CH_2N(CO)_2C_6H_4 \rightarrow$

$C_6H_5.NH{-}N = C(COOR).CH_2CH_2CH_2N(CO)_2C_6H_4 \rightarrow$

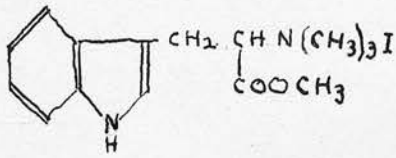


By heating with methyl iodide in methyl alcohol containing potassium hydroxide, the indole-3-ethylamino-2-carboxylic acid was converted into the methyl ester of its N-iodotrimethyl derivative, m.p. 249°.



which was found not to be identical with the isomeric compound, m.p. 195°, prepared from abrinine (mixed m.p.).

At this stage the investigation had to be interrupted, owing to the appearance of Hoshino's paper on this subject. This proved that abrinine was in fact not indole-3-(N-methyl) ethylamino-2-carboxylic acid, but N-methyltryptophane, and that the compound m.p. 195° has really the constitution:



Hoshino's formula also explains the failure to obtain a fluorescing compound from the alkaloid and is in general harmony with the observations recorded above. The failure to obtain an immediate Adamkievič reaction emphasises the importance of not attaching too much weight to colour reactions of this type.

I. VITEXIN:

Introduction.

Theoretical.

Experimental.

Introduction.

In 1898 Sir Thomas Wardle sent A.G. Perkin a small sample of a New Zealand dyewood "Puriri" (Vitex littoralis) asking his opinion as to its commercial value. The hydrolysis of the aqueous extract of the wood yielded a yellow, crystalline compound, for which Perkin (J.C.S., 1898, 1019) proposed the name vitexin.

Vitex littoralis is a large tree which grows only in the northern portion of the North Island of New Zealand. Perkin (loc. cit.) extracted the wood, previously ground up, with boiling water. On evaporating the light brown extract, an almost black, treacly substance was left. This residue was digested with alcohol, filtered and the alcohol evaporated, leaving an orange coloured vitreous residue, a glucoside, the aqueous solution of which was hydrolysed by means of dilute hydrochloric acid. The viscous yellow product so obtained was digested with boiling alcohol. Different impurities, including a substance called "homovitexin" were extracted with alcohol, the vitexin being left undissolved in the form of a crystalline powder.

Vitexin/

Vitexin is a yellow micro-crystalline compound, m.p. 264-265° (Perkin), 260° (Barger), insoluble in most of the usual organic solvents; its solubility in alcohol or acetic acid is so small that these solvents cannot be used for crystallisation. It is readily soluble in hot pyridine but does not separate on cooling. For purification it is dissolved in pyridine and an equal volume of water is added. On shaking the mixture with petroleum ether, vitexin separates in micro-crystalline form. Vitexin is readily soluble in dilute NaOH, Na_2CO_3 or NH_4OH, but is readily reprecipitated on acidification. With a trace of ferric chloride it gives a reddish brown coloration.

Vitexin has dyeing properties similar to those of apigenin. However, its use as a dyestuff was restricted to New Zealand, and to-day when the synthetic dyestuffs are available at astonishingly low prices, natural dyestuffs, even with much better qualities than vitexin, are unable to compete.

According to E.H. King (A.P. 1,720,278 and 1,720,279) vitexin can be used in chemotherapy. In quinine-haematoxylinate - a medicament used in the/

the therapy of diseases such as carcinoma - the haematoxyline can be replaced by brasilin, quercetin or vitexin without affecting the therapeutic activity of the compound.

From his analytical results Perkin (loc. cit.) suggested the formula $C_{15}H_{14}O_7$ or $C_{17}H_{16}O_8$. Vitexin forms an acetyl derivative, m.p. 251-256°, to which he assigned the formula $C_{15}H_9O_7(C_2H_3O)_5$ or $C_{17}H_{10}O_8(C_2H_3O)_6$. Attempts to methylate the hydroxy groups of vitexin were unsuccessful. On fusion with alkali phloroglucinol and p-hydroxybenzoic acid were obtained and a strong smell of acetic acid was observed. On hydrolysis with boiling 50% KOH phloroglucinol, p-hydroxyacetophenone and a small amount of p-hydroxybenzoic acid were obtained. The action of alcoholic potash yielded p-ethoxybenzoic acid. No pure compound could be isolated from the product obtained by heating vitexin with concentrated nitric acid. On heating vitexin with dilute nitric acid dinitroparahydroxybenzoic acid, picric acid and a nitro compound, m.p. 239-241° were obtained. To the latter Perkin assigned the formula $C_{15}H_6O_5(NO_2)_4$, which is identical with the formula of tetranitroapigenin.

Tetra/

Tetranitroapigenin was prepared (A.G. Perkin, J.C.S., 1900, 416) and the similarity of the two melting points in addition to the identical formulae led Perkin to the conclusion that tetranitrovitexin and tetranitroapigenin were identical. However no statement as to the m.p. of the mixed substances can be found in his paper. Having apparently established a relationship between vitexin and apigenin, Perkin expressed the view that vitexin is a "very stable glucoside" of apigenin, the relationship being expressed by the following equation:

$$\underset{\text{vitexin}}{C_{21}H_{20}O_{10}} + H_2O = \underset{\text{apigenin}}{C_{15}H_{10}O_5} + C_6H_{12}O_6$$

This conclusion was found to be consistent with the analytical results. Indeed figures calculated for $C_{21}H_{20}O_{10}$ are closer to the figures found by combustion than do those calculated for $C_{15}H_{14}O_7$ or $C_{17}H_{16}O_8$ as is shown in the following table:

$C_{15}H_{14}O_7$	requires	C = 58.82%,	H = 4.57%.
$C_{17}H_{16}O_8$	"	C = 58.62%,	H = 4.59%.
$C_{21}H_{20}O_{10}$	"	C = 58.33%,	H = 4.62%.

Found for vitexin: C = 58.47, 58.63, 58.69%.
H = 4.69, 4.71, 4.58%.

$C_{15}H_{14}O_7(C_2H_2O)_5$ requires C = 58.14%, H = 4.65%

$C_{17}H_{16}O_8(C_2H_2O)_6$ " C = 58.00%, H = 4.66%

$C_{21}H_{20}O_{10}(C_2H_2O)_7$ " C = 57.85%, H = 4.68%

Found for acetylvitexin C = 57.51%, H = 4.80%.

A few years later G. Barger (J.C.S., 1906,1210) obtained by hydrolysis of the glucoside saponarin a small amount of a yellow compound, which he recognised as identical with vitexin. In contrast to the glucoside present in Vitex littoralis, saponarin occurs in very many plants (see Klein, Handbuch d. Pflanzenanalyse, 3, 1449). Like starch it gives a blue coloration with iodine. The presence in Gagea lutea of a compound giving a blue coloration with iodine was first observed by Sanio (Botanische Zeitung, 1857, 15, 420) who actually considered the compound responsible to be starch, but in 1906 G. Barger isolated the glucoside from Saponaria officinalis. On hydrolysis glucose and an amorphous substance, saponaretin, were obtained as main products, besides vitexin in a yield not exceeding 13% of the total possible. To both vitexin/

vitexin and saponaretin was assigned the same empirical formula, $C_{15}H_{14}O_7$. The identity of vitexin from Vitex littoralis with that from saponarin was shown by the m.p. of the mixed substances.

Mol. wt. found 283.

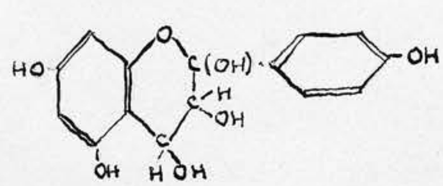
$C_{15}H_{14}O_7$ requires 306.

$C_{21}H_{20}H_{10}$ " 432.

These observations agreed with the view of Barger that vitexin is not a stable glucoside of apigenin. He thought it more probable that vitexin differs from apigenin by the elements of two molecules of water.

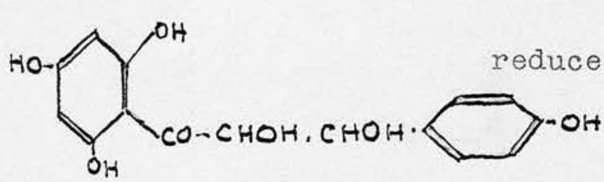
$$\underset{\text{apigenin}}{C_{15}H_{10}O_5} + 2H_2O = \underset{\text{vitexin}}{C_{15}H_{14}O_7}$$

and suggested the two formulae:



reduced flavanone.

or



reduced chalkone.

The/

The formation of parahydroxyacetophenone on hydrolysis of vitexin can be explained according to Barger by the loss of one mol H_2O, yielding the compound $CH_2:CH(OH).C_6H_4OH$. This compound is the enol form of parahydroxyacetophenone. The reaction is analogous to the formation of laevulic acid from dextrose.

THEORETICAL.

In the present work attempts were made to prove the relationship between vitexin and apigenin. Perkin (loc. cit.) claimed that tetranitrovitexin and tetranitroapigenin were identical, but the absence of a m.p. of the mixed substances and a wide discrepancy between the theoretical and experimental amounts of hydrogen cast some doubt on this result. Thus on p. 1026 (J.C.S., 1898) the following data are supplied:

Analysis of tetranitrovitexin.

Gm. subst.	gm. H_2O	gm. CO_2	% H	% C
0.1097	0.0220	0.1602	2.22	39.82
0.1227	0.0225	0.1815	2.03	40.34

$C_{15}H_6O_5(NO_2)_4$ requires C, 40.00; H, 1.35.

In order to check Perkin's analysis, nitrovitexin was prepared (Cf. Perkin, loc. cit.). The crude product melted at 251° and after crystallisation from nitrobenzene followed by boiling with alcohol, the melting point was lowered. It would/

would appear that boiling nitrobenzene (b.p. 211°) slightly decomposes the compound. After various solvents had been tried, it was found that dioxane is ideal for crystallising nitrovitexin. The m.p. was raised to 257° (Perkin 239-241°) and the new analysis gave results required for the formula of tetranitroapigenin ($C_{15}H_6O_5(NO_2)_4$). The mixed m.p. of the two compounds gave no depression, completely establishing the identity of tetranitroapigenin with tetranitrovitexin.

Attempts were made to obtain apigenin directly from vitexin. Experiments with dehydrating agents - such as phosphorus pentoxide, potassium hydrosulphate, a mixture of sulphuric and acetic acids, formic acid - were not successful. Better results were obtained with anhydrous oxalic acid at 140°. The reaction product was heated in a high vacuum and a minute amount of a yellow compound sublimed. Since the reaction mixture never became entirely liquid, it was desirable to find a more suitable method. The best yield of the dehydrogenation product was obtained when vitexin was heated with a mixture of oxalic and formic acids 10:1, b.p. 125°, but in no case/

case was the yield satisfactory.

Apparently the same compound was obtained by heating vitexin with formic acid in a sealed tube at 140° or boiling it for half an hour with acetic acid and zinc chloride. Unfortunately the yield was always very poor.

The compound could not be crystallised from any solvent and had to be purified by sublimation. A few mg. were acetylated. The product of the acetylation was found to be a mixture of two substances. The minute quantity available was not sufficient for further purification. The preparation of the dehydration product by this method being a very wasteful process, further experiments in this direction were abandoned.

The distillation with zinc dust in a high vacuum yielded a minute quantity of a substance which surprisingly contained a large amount of oxygen and was soluble in sodium carbonate, being thus a polyphenol. On analysis it gave the formula $C_{15}H_{12}O_6$, which contains one H_2O less than vitexin. On acetylation a further mol of water was split off yielding/

yielding the triacetyl derivative m.p. 180° of a compound $C_{15}H_{10}O_5$. This compound gave no depression of the m.p. with triacetyl apigenin m.p. 181-182°.

This is another proof of the close relationship between apigenin and vitexin, supporting the results of Perkin already mentioned.

Attempts were made to oxidise the aliphatic hydroxyls of vitexin to ketones or aldehydes or to obtain any degradation product containing all three of the aliphatic carbon atoms. Oxidation with Fehling's reagent yielded phloroglucinol and p-hydroxyacetophenone besides an amorphous acid; potassium ferricyanide gave p-hydroxybenzoic acid; hydrogen peroxide yielded p-hydroxybenzoic acid and a minute amount of hydroquinone. A large quantity of ammoniacal silver nitrate was reduced by vitexin; the oxidation product could not be isolated. Vitexin did not react with lead tetra-acetate in acetic acid, owing possibly to the insolubility of vitexin in that solvent.

It would be important to obtain the methylated vitexin/

vitexin since this compound would probably not decompose as readily by either hydrolysis or oxidation. However vitexin refused to yield crystalline methylation compounds (cf. Barger, loc.cit).

Vitexin is optically active. Its acetyl derivative has $[\alpha]_D^{20} = -60^\circ$. This observation is compatible with either of the two formulae suggested by Barger (loc. cit.) and does not therefore assist in deciding between the two formulae.

Both formulae have six hydroxyls whilst acetyl estimations of Barger and of Perkin found only five acetyl groups. The C and H contents of acetylvitexin cannot decide between the formulae of the penta and hexa acetyl compounds.

$C_{15}H_{14}O_7(C_2H_2O)_5$ requires C, 58.14%; 4.65%.

$C_{15}H_{14}O_7(C_2H_2O)_6$ " C, 58.06%; 4.66%.

The large discrepancy between the acetyl determinations of Perkin and of Barger on the one hand and recent micro-analysis on the other, makes it possible that these results are not conclusive, and that vitexin has six acetyl groups.

A.G. Perkin/

A.G. Perkin	42.61, 42.80% acetyl
G. Barger	42.40, 40.05%
H. Roth	35.15, 35.28%

$C_{15}H_{14}O_7(C_2H_2O)_5$ requires 40.69%:

$C_{15}H_{14}O_7(C_2H_2O)_6$ " 45.16%.

If vitexin were either a reduced chalkone or a reduced flavanone derivative it would be expected to be colourless. However the fact that vitexin is yellow is extremely difficult to explain.

The fact that the compound $C_{15}H_{12}O_6$ loses readily water on boiling with acetic anhydride suggests a ring closure and makes it probable that it has the chalkone formula. This indicates that vitexin is also probably an open chalkone derivative and it may have the constitution suggested by Barger (cf. p. 13).

Finally it should be mentioned that an improved method has been discovered for the preparation of crystalline vitexin. Allowing a solution in hot 40-50% pyridine to cool, it is obtained in the form of glistening pale yellow crystals, (m.p. 263°) which have a slight greenish tint.

EXPERIMENTAL.

Preparation of Vitexin

The starting material was a dark sticky residue of an alcoholic extract of the wood of Vitex littoralis. 200 Gm. of this extract were digested with a small amount of water, and this action was repeated on the residue three times. The amount of the undissolved substance was 22.5 gm.

On adding more water to the aqueous solution a tarry substance separated. After decantation the solution was evaporated to dryness in vacuo. A golden yellow amorphous compound was obtained Yield 96 gm. This substance, a mixture of glucosides, was hydrolysed according to the method of Perkin (loc. cit., p.1020). 96 Gm. of the glucoside were heated for 2 hours with stirring with 900 c.c. 2% HCl. The following day the acid liquid was removed by decantation and the tarry residue was washed several times with boiling alcohol, so converted into a micro-crystalline mass, which was filtered. Vitexin thus prepared melted at 246°. It was recrystallised by dissolving in hot 40% aqueous pyridine. On cooling, vitexin separated in the form of glistening pale yellow plates/

plates with a greenish tint. The crystals were filtered and washed with hot glacial acetic acid and then with hot methyl alcohol. The m.p. was 263°; it was not raised by two further crystallisations. The analysis gave the following results:

2.947 mg. dried at 100° in high vacuum, loss of weight 0.039 = 1.32%.

2.908 mg. gave 6.240 mg. CO_2, 1.240 mg. H_2O; C, 58.52%; H, 4.77%.

$C_{15}H_{14}O_7$ requires C, 58.82%; H, 4.57%.

Acetylvitexin

0.170 Gm. of vitexin was heated for 6 hours with ten times its weight of acetic anhydride. After cooling absolute alcohol was added. The acetylvitexin crystallised overnight in large white stout prisms. Yield 0.181 gm., 63%. For crystallisation it was dissolved in hot glacial acetic acid, from which it separated after the addition of alcohol. A sample crystallised twice from glacial acetic acid-alcohol, twice from glacial acetic acid and finally from dioxane to which a few drops of petroleum ether (b.p. 60-80°) were added, had the m.p. 251-256° and gave the following/

following analytical figures:

4.142 mg. gave 1.765 mg. H_2O, 9.26 mg. CO_2;
C, 57.94%; H, 4.77%.

4.483 mg. gave 1.955 mg. H_2O, 9.28 mg. CO_2;
C, 57.73%; H, 4.88%.

$C_{15}H_{14}O_7(C_2H_2O)_5$ requires C, 58.14%; H, 4.65%.

Dehydration of Vitexin.

On heating 0.5 gm. vitexin with 20 c.c. of a 33% aqueous solution of oxalic acid for 6 hours, no reaction occurred; vitexin remained undissolved.

0.2 Gm. vitexin was heated with 2 gm. anhydrous oxalic acid for half an hour to 140°. The mixture was boiled with water to dissolve the oxalic acid. After filtration a yellowish green amorphous substance was obtained, which was readily soluble in alcohol or acetone, but all efforts to crystallise it were unsuccessful. On sublimation in the high vacuum a pale yellow compound was obtained in a very small yield at 245-255°. The residue charred.

0.1 Gm. vitexin was heated in vacuo with

$KHSO_4$ /

$KHSO_4$; the substance charred.

0.1 Gm. vitexin was heated with P_2O_5 in vacuo. Frothing occurred and the substance charred.

0.1 Gm. vitexin was heated with an equal volume of concentrated H_2SO_4 and purified glacial acetic acid; charring again occurred.

0.1 Gm. vitexin was heated with 5 c.c. formic acid. The substance dissolved. After an hour water was added, the greenish yellow precipitate filtered, washed, dried and sublimed in the high vacuum. No sublimate was obtained.

Oxalic acid and formic acid, 1:2, were heated for an hour with vitexin. The b.p. of the mixture was 110°. On working up as above a trace of the sublimate was obtained.

Vitexin was heated for an hour with oxalic acid and formic acid 2:1, b.p. 116°. On working up a small amount of the sublimate was obtained.

Vitexin heated with oxalic acid-formic acid 3:1, b.p. 118°, yielded sublimate.

Vitexin heated with oxalic acid-formic acid 6:1, b.p. 121°, yielded sublimate.

Vitexin heated with oxalic acid-formic acid 10:1, b.p. 125°, yielded sublimate.

Vitexin/

Vitexin heated with oxalic acid-formic acid 20:1, b.p. 133°, yielded sublimate, somewhat less than with the mixture 10:1.

1 Gm. vitexin was heated with formic acid to 140° in a sealed tube, and worked up as above. The sublimate was obtained.

1 Gm. vitexin was heated for 2 hours with $ZnCl_2$ in acetic acid. On diluting with water a yellow precipitate was obtained, which was very readily soluble in alcohol and could not be crystallised. On heating in the high vacuum a small amount of a sublimate was obtained at 245-255°.

Acetylation of the product of the dehydration.

15 Mg. of the resublimed dehydration product of vitexin were heated with 1 c.c. acetic anhydride and a trace of pyridine for half an hour. Water was added and the precipitate collected and crystallised from alcohol. Two fractions were separated, one being readily, the other slightly soluble in hot alcohol. However the small quantity of/

of acetyl products available was not sufficient for further purification.

Attempts to prepare a keto derivative.

77 Mg. vitexin were heated on the water bath with 41 mg. p-nitrophenylhydrazine in 2 c.c. 50% acetic acid for one hour. On cooling, orange yellow crystals, m.p. 260° were obtained, but these appeared to be unchanged vitexin because on acetylation they yielded the characteristic penta-acetylvitexin m.p. 251-256°.

Optical activity.

200 Mg. acetylvitexin were dissolved in glacial acetic acid so that the resulting volume was 2 c.c. The solution was transferred to a dm. tube and examined in polarised light. Using the D line of Na, the observed rotation was -6°; thus $[\alpha]_D^{20}$ = 60°. (This is probably correct within about 1°).

Oxidation/

Oxidation with hydrogen peroxide.

1 Gm. vitexin was dissolved in 4 c.c. about 2N NaOH, and 6 c.c. 30% H_2O_2 and 3 c.c. water were added. The following day PtO_2 was added to decompose the excess of H_2O_2, and the solution was acidified. The crystalline precipitate was filtered, washed and dried, m.p. 246°; it gave no m.p. depression with vitexin. The mother liquor was extracted several times with ether. The ethereal extract was shaken first with aqueous $NaHCO_3$ (a) and then with aqueous Na_2CO_3 (b). The ether was evaporated to dryness but yielded practically no residue.

(a) The $NaHCO_3$ solution was acidified, extracted with ether, the ether dried over Na_2SO_4 and evaporated to dryness. The crude product melted at 210° and gave no depression with p-hydroxybenzoic acid (m.p. 213°).

(b) The Na_2CO_3 solution was acidified and extracted with ether. After drying over Na_2SO_4 and evaporating the ether, a brownish residue was obtained, which was extracted with boiling benzene. On cooling a small amount of a crystalline compound was obtained. 5 Gm. vitexin yielded 40 mg. The yield/

yield of this compound depended much on the concentration of the alkali. If 5 c.c. 2N NaOH were taken in place of 4 c.c., no yield of the oxidation product was obtained. The m.p. was 168° and was raised by sublimation to 173°. This compound gave no depression of the m.p. with hydroquinone.

20 Mg. were heated with 1 c.c. acetic anhydride and a trace of pyridine. After precipitation with water the acetyl product was crystallised from alcohol, m.p. 123°; no depression with acetyl hydroquinone.

Oxidation with Fehling's solution.

0.5 Gm. of vitexin was refluxed with 50 c.c. of Fehling's solution for 45 minutes. After cooling, the cuprous oxide was removed by filtration through asbestos and the solution was acidified with HCl. A crystalline substance separated and was recrystallised from water. It proved to be acid potassium tartrate derived from the reagent. The mother liquor was then extracted with ether. The ethereal extract was shaken with aqueous $NaHCO_3$. The $NaHCO_3$ /

$NaHCO_3$ solution was acidified and extracted with ether. A red amorphous compound was obtained, which did not sublime, was very soluble in alcohol, and attempts to crystallise it were not successful. A small portion of the ethereal extract left after shaking with $NaHCO_3$ was extracted with NaOH. No residue was left in the ether from which it appeared that all the compound present must have been phenolic in nature. This being the case, the main portion was dried over Na_2SO_4 and the ether evaporated. On extracting the residue with benzene a separation into two parts was effected. One substance was but slightly soluble in benzene, gave a blue-violet coloration with ferric chloride, pinewood dipped into concentrated HCl was coloured red by the substance. The m.p. being 209°, the substance was recognised as phloroglucinol. It gave no depression with phloroglucinol.

On concentrating the benzene solution the second substance crystallised out. It was finally purified by subliming in the high vacuum. The m.p. was 106°, the same as that of p-hydroxyacetophenone (previously isolated from vitexin by hydrolysis/

hydrolysis with 50% KOH) with which it was obviously identical.

Oxidation with potassium ferricyanide.

0.5 Gm. vitexin was dissolved in 7.5 gm. 33% NaOH and a 25% aqueous solution of $K_3Fe(CN)_6$ was added in small portions. Roughly the amount, corresponding to 15 atoms of oxygen (15 gm. $K_3Fe(CN)_6$) was used, before the excess of the reagent could be detected by means of the Prussian blue reaction with $FeSO_4$. The solution was acidified and extracted with ether. After drying over Na_2SO_4 and evaporating the ether, a crystalline substance was obtained, which was purified by sublimation. The m.p. was 209°; no depression of the m.p. with p-hydroxybenzoic acid was observed.

Action of lead tetra-acetate on vitexin.

1 Gm. vitexin was heated with 1.44 gm. lead tetra-acetate (1 mol.) in acetic acid. Vitexin did not dissolve and apparently no reaction occurred.

Oxidation with ammoniacal silver nitrate.

1 Gm. vitexin was heated on the water bath with an excess of ammoniacal silver nitrate. A silver mirror appeared very soon but the reaction was completed only after several hours. The large amount of silver was filtered off and the mother liquor was acidified and extracted with ether. On evaporating the dry ethereal extract practically no residue was obtained.

Attempts to methylate vitexin.

0.5 Gm. crystalline vitexin was suspended in 2.5 c.c. absolute alcohol and 0.47 c.c. $(CH_3)_2SO_4$ and then 0.28 gm. potassium hydroxide dissolved in 0.25 gm. water were added. After a short time the same quantities of $(CH_3)_2SO_4$ and potassium hydroxide were again added. The clear solution was acidified and extracted with ether. The ethereal extract was separated from acid decomposition products by shaking with aqueous $NaHCO_3$. The ether was then dried over Na_2SO_4 and evaporated. A small quantity of a canary yellow amorphous compound was obtained. It was soluble in NaOH, alcohol, acetone, and separated in an amorphous condition from hot water on cooling.

It/

It was heated with methyl iodide and silver oxide for five hours, the Ag_2O removed by filtration and the CH_3I evaporated. The amorphous residue could not be crystallised.

From the aqueous mother liquor of the methylation mixture a gelatinous substance separated overnight. This was filtered and was found to dissolve readily in alcohol. On cooling, a hot aqueous solution a gel was formed, even if the concentration of the solution was less than ½%.

Attempts to methylate vitexin by treating with diazomethane or by boiling a suspension in methyl iodide with Ag_2O for several days on the water bath were unsuccessful.

Nitration of vitexin.

Using the method of Perkin (J.C.S., 1898, 1024), 1.33 gm. vitexin were heated with 33 c.c. 15% nitric acid. The substance dissolved slowly to a clear solution, but after about half an hour a pale yellow crystalline powder separated in small quantity. This compound was filtered and washed with a little water. (On cooling the reaction mixture or on adding a little water to it, the p-hydroxydinitro- benzoic/

benzoic acid may also separate. This acid can be removed by washing the mixture of the nitro compounds with boiling alcohol in which solvent tetranitrovitexin is insoluble). The dry tetranitrovitexin was then dissolved in boiling dioxane, from which it separated in yellow prisms m.p. 257°. On recrystallisation the m.p. was not raised. The analysis gave the following results:

4.193 mg. subst. gave 0.58 mg. H_2O, 6.20 mg. CO_2.
C, 40.33%; H, 1.55%.

4.379 mg. subst. gave 0.64 mg. H_2O, 6.50 mg. CO_2.
C, 40.48%; H, 1.64%.

	p.	t°	v	
2.068 mg.	752	23	0.233	N, 12.86%.
2.999 mg.	752	22	0.331	N, 12.68%.

$C_{15}H_6O_5(NO_2)_4$ requires C, 40.00%; N, 12.44%; H, 1.33%

Tetranitroapigenin was prepared (cf. Perkin, J.C.S., 1900, 77, 416); it gave with tetranitrovitexin no m.p. depression, proving the identity of the two substances.

Sublimation/

Sublimation with zinc dust.

1 Gm. vitexin was mixed with 10 gm. zinc dust and the mixture was filled into several small test tubes. A little asbestos was finally placed over the mixture and the tubes were heated in a high vacuum in a metal bath. Above 310° a yellow sublimate appeared. The main part sublimed at about 350-360° fairly slowly. The tubes were opened and the yellow substance collected and twice resublimed. The temperature of the bath was kept at 260-270° and the substance sublimed very slowly, a great part of it, however, being charred. Thus, after the second sublimation about 5 mg. were obtained. The substance was readily soluble in aqueous Na_2CO_3.

Analysis:

1.960 mg. subst. gave 4.49 mg. CO_2; 0.67 mg. H_2O;

C = 62.48%; H = 3.82%.

$C_{15}H_{12}O_6$ requires C = 62.50%; H = 4.16%.

About 20 mg. of the sublimate were heated for 3 hours with 1 c.c. acetic anhydride. After cooling, water was added and the precipitate filtered off, washed, dried and crystallised from alcohol, m.p. 180° /

180°. Yield 11 mg.

On analysis the following figures were obtained:

3.451 mg.	1.20 mg. H_2O; 8.09 mg. CO_2	
		C = 63.94%; H = 3.89%.
3.695 mg.	1.34 mg. H_2O; 8.64 mg. CO_2.	
		C = 63.77%; H = 4.06%

$C_{15}H_{10}O_5(C_2H_2O)_3$ requires C = 63.63%; H = 4.04%

The substance gave no depression of the m.p. with triacetyl apigenin m.p. 181-182°, establishing the identity of the two substances.

II. VERATRINE.

Introduction.

Theoretical.

Experimental.

INTRODUCTION.

The mixture of the veratrine alkaloids first obtained by Meissner (1818) from Veratrum sabadilla, contains mainly three substances.

(a) Cevadilline was isolated by Wright and Luff (J.C.S., 1878, 338) who assigned to it the formula $C_{34}H_{53}NO_8$. This alkaloid might be identical with the compound named sabadilline,mentioned by Weigelin (Untersuchungen über die Alkaloide der Sabadillsamen, Dissert. Dorpat. 1871). The hydrolysis yielded a base, cevilline $C_{29}H_{47}NO_7$ and α-methylcrotonic acid.

(b) Veratridine was first isolated by Couerbe (Ann., 1834, 9, 111) and to it was assigned the formula $C_{37}H_{53}NO_{11}$ by Wright and Luff (loc. cit.) who obtained by hydrolysis veratric acid and a base verine, $C_{28}H_{45}NO_8$. Investigations of Blount (J.C.S. 1935, 122) however proved the identity of verine with cevine $C_{27}H_{43}NO_8$.

(c) Cevadine or "veratrinum puriss. cryst." was first isolated by Merck (Ann., 1855, 95, 200) and/

and to it was assigned the formula $C_{32}H_{49}NO_9$ by Wright and Luff (loc. cit.). The same authors obtained by hydrolysis α-methyl crotonic acid and a base cevine, $C_{27}H_{43}NO_8$. They suggested that the methyl crotonic acid was the "trans" form - tiglic acid. However Bosetti (Arch. Pharm., 1883, 221, 81) and Ahrens (B., 1890, 23, 2700) found the "cis" form - angelic acid, Frankforter (Amer. Chem. J. 1898, 20, 361) found again tiglic acid, whilst Freund and Schwarz (B., 1899, 32, 800) isolated both tiglic and angelic acids. It is known that tiglic acid cannot be converted into angelic acid, the opposite reaction however goes very readily on heating with aqueous or alcoholic alkali or aqueous hydrochloric acid, but not in alcoholic hydrochloric acid (Horst, Chem. Ztg., 1902, 26, 334; C., 1902, I, 1155). The same author obtained tiglic acid by hydrolysis of cevadine with alcoholic hydrochloric acid, and therefore it was accepted that cevadine contains tiglic acid and not angelic acid.

Very little is known about the structure of cevine. The base was crystallised first by Freund and/

and Schwarz (loc. cit.). Cevine is a white, crystalline base, m.p. 205° (previous sintering). It contains 3½ mol crystallisation water. It is readily soluble in alcohol, acetone, chloroform or benzene. In contrast to the amorphous anhydrous base, the crystals are but slightly soluble in water or ether. On heating a cold aqueous solution of cevine it becomes turbid. Cevine is laevorotatory, $[\alpha]_D^{17} = -15.36°$ in methyl alcohol. On heating with acids it gives a deep red coloration which turns green when made alkaline and which disappears when diluted. Cevine reduces Fehling's solution and ammoniacal silver nitrate. Cevine is precipitated from solution in acids by ammonia but on heating it liberates ammonia from ammonium salts. It forms a crystalline methiodide m.p. 257°, chloroaurate (decomp. 162°) and hydrochloride m.p. 247°.

Freund obtained a dibenzoyl derivative (B., 1904, 37, 1946) and suggested the presence of two hydroxyl groups. Frankforter (loc. cit.) found one methoxy group but later Freund (loc. cit.) proved that cevine contains neither a methoxy nor an/

an N-methyl group. Two of the oxygen atoms are present in a lactone group (Macbeth and Robinson, J.C.S., 1922, 1571).

By dry distillation β-picoline was obtained (Ahrens, loc. cit.; Frankforter, loc. cit.), the lime distillation yielded β-pipecoline and β-picoline (Ahrens,loc. cit.), whilst the distillation with soda lime in a hydrogen stream yielded a base,the properties of which indicated that it was coniine (Macbeth and Robinson, loc. cit.).

By treating cevine with strong hydrogen peroxide, Freund (loc. cit.) obtained a base, which had one oxygen more than cevine. Cevinoxide is of the type of the ammonium oxides $R_3N:O$, and by reduction with sulphur dioxide it can be converted into cevine. Since cevine contains no N-methyl group, Freund suggested that the nitrogen atom belongs to two rings. Cevinoxide has a sharper m.p. (278°) than cevine so that it may be used for identifying the alkaloid.

On the selenium dehydrogenation of cevine a base, cevanthridine $C_{23}H_{25}N$ m.p. 207°, probably a phenanthridine derivative (Blount, J.C.S., 1935, 122/

122) and cevanthrol $C_{17}H_{16}O$ m.p. 197-198°, probably a phenanthrene derivative with a phenolic hydroxyl, were obtained . According to X-ray examinations this hydroxyl would be in the position 3. (Blount and Crowfoot, J.C.S., 1936, 414).

Cevadine is toxic. It paralyses the central nervous system, is locally irritating and is a very effective sneezing powder. The physiological properties of cevine are similar but less pronounced.

THEORETICAL

In the present investigation several oxidation experiments were carried out. Satisfactory results were obtained only if the experiment was carried out in the cold and in the absence of heavy metallic radicals. By oxidation of cevine with lead tetra-acetate a lead complex, with ferric chloride an iron complex, with potassium ferricyanide a ferrocyanide complex, with chromic acid a chromium complex was obtained. This behaviour of cevine suggested the presence of many hydroxyl groups. Other polyhydroxyl compounds such as inositol show a similar tendency to form a complex compound with heavy metal salts (cf. Windaus, B., 1907, 40, 802; Smirnov, Sborn. Rabot Chim. Tabak Bull., 1935, No. 125, 87). Since anhydrous cevine cannot be obtained in a crystalline form, the determination of the active hydrogens was carried out with cevine oxide. It yielded six active hydrogens. From this follows, that with the exception of the two oxygen atoms present in a lactone ring, all the oxygen atoms of cevine have a hydroxylic function. The fact that cevine forms only/

only a dibenzoyl or a diacetyl derivative, could possibly be explained by steric hindrance.

The red amorphous compound produced from cevine by the action of hydrochloric acid can also be obtained by means of many other acids. When cevine is refluxed with acetic or formic acid, a deep blood-red coloration develops gradually. On heating cevine in a toluene solution with phosphorus pentoxide a similar red compound was produced. On making alkaline, it turned green. The green base could be isolated by continuous extraction with ether but could not be crystallised. By oxidation with Caro's acid in the cold, a brown amorphous compound was obtained, which did not crystallise. Concentrated nitric acid gave an uncrystallisable pale yellow gum.

Oxidation experiments in alkaline medium - such as Fehling's solution, ammoniacal silver nitrate or potash fusion - yielded usually brown amorphous substances, insoluble in ether. A similar compound was obtained by the oxidation with potassium permanganate in acetone solution. It was found that the products obtained from the potash fusion and from the potassium permanganate oxidation/

oxidation contain a non-basic nitrogen. On hydrolysis a base was split off which was volatile with the vapours and turned red litmus paper blue. This result seems to indicate the presence in the oxidation product of a lactam group.

The byproduct of the potash fusion was a minute quantity of a substance, very probably oxalic acid, whilst a small amount of an acid obtained at the potassium permanganate oxidation as a white amorphous powder could not be crystallised.

On oxidation with hydrogen peroxide - beside cevine oxide, acetic acid and a syrupy acid containing a non-basic nitrogen - a crystalline base was obtained in a good yield by extraction with ether. This base m.p. 153° (compound A in the experimental part) has the formula $C_{19}H_{31}O_6N$ or $C_{20}H_{33}O_6N$. The number of the active hydrogens indicates that five out of the six oxygen atoms are present as hydroxyls; the sixth oxygen may be expected to be in a ketone group. However no keto derivative could be isolated. Compound A is coloured red when heated with acid and in its properties it closely resembles cevine. A side chain determination by the Kuhn-Roth method indicates that compound A has one side chain.

On/

On oxidising cevine with ozone another crystalline compound was obtained which however is still under investigation.

The present investigations show in first place the difficulty of the problem, due primarily to the large number of hydroxyls present in the cevine molecule. The fact that non-basic ether insoluble amorphous compounds are very readily formed, makes it difficult to obtain well defined decomposition products. However the fact that these substances yield bases on hydrolysis may throw some light on the constitution of cevine since it indicates the probable presence of a lactam group -CONRR'. This lactame group may have been derived from -CHOH.NRR' in the alkaloid.

On writing the introduction to this part of the present thesis, the idea suggested itself that a substance m.p. 42° obtained by earlier experiments from cevadine by zinc dust distillation, might be identical with angelic acid. At that time the experiment was repeated with cevine and since the same substance could not be obtained again, it was considered/

considered to be an impurity of the cevadine and was not further investigated. From the fact that under circumstances in which angelic acid is not converted into tiglic acid the latter was obtained from cevadine, Horst (loc. cit.) concluded that tiglic acid and not angelic acid was present. This fact by no means explains why other authors found angelic acid which cannot be obtained from tiglic acid by any known reaction. Therefore it seemed to be of interest to investigate the substance, m.p. 42°. Unfortunately the shortage of the time did not allow a micro-analysis, nor could any genuine angelic acid be purchased. However the identity of the substance, m.p. 42° with angelic acid was shown in the following way. The substance gave a lowering of the m.p. with tiglic acid. By titration its equivalent weight was found to be 95 (angelic acid requires equiv.wt. 90). The substance (7.8 mg.) used for the titration was then heated in a 30% sodium hydroxide solution on the water bath to convert the hypothetical angelic acid into tiglic acid. A crystalline substance was obtained/

obtained from this reaction but its amount was just enough to take one m.p. of the crude product. The melting point was 56-57° (angelic acid 45°, tiglic acid 64°).

The mixed m.p. was examined in the same m.p. tube, by adding a crystal of tiglic acid and mixing the two substances by melting. The mixture crystallised immediately on cooling and no depression of the m.p. (64°) was observed.

This is an indirect proof that the substance obtained from cevadine by zinc dust distillation was identical with angelic acid.

Since angelic acid is converted into tiglic acid by refluxing or by heating with aqueous and alcoholic alkali and aqueous acids, it is possible that the observation of Horst (loc. cit.) that the conversion does not proceed in alcoholic hydrochloric acid was an experimental error.

EXPERIMENTAL.

Preparation of Cevine.

Crystalline cevadine (Veratrinum puriss. cryst., Merck) was hydrolysed according to the method described by R. Robinson and Macbeth (loc. cit.). 20 Gm. cevine were heated in 75 c.c. absolute alcohol and 100 c.c. of an absolute alcoholic solution of potassium hydroxide, saturated at the boiling point were added, and the mixture refluxed on a water bath for half an hour. The liquid was inocculated with a few crystals of the potassium salt of cevine, which then separated very gradually. The crystals were filtered off, washed with a little alcohol and much ether and pressed on a porous plate. The snow-white substance was dissolved in water, filtered and CO_2 passed into the solution. After a few minutes cevine precipitated in the form of a white paste. After standing in contact with cold water for 4-5 hours the amorphous paste changed into a crystalline compound. From the mother liquor on standing a further quantity of cevine separated in well developed crystals, m.p. 205° (previous sintering/

sintering). The total yield from 180 gm. cevadine was 120 gm. cevine, 68.9%.

Oxidation with chromic acid.

On adding a solution of chromic acid to a solution of cevine, both in acetic acid, a brown precipitate (a complex salt) is formed, which gradually disappears. After evaporating the acetic acid in vacuo, the residue was dissolved in dilute sulphuric acid, and extracted with ether, and then with chloroform; but no oxidation product could be isolated. This experiment was repeated with various proportions of chromic acid and at different temperatures, but the same negative result was always obtained.

Potash fusion.

Cevine did not dissolve when fused with potash. Above 240° it formed a dark brown paste floating on the top of the fusion and a gas (probably conine) was developed at a very slow rate. Even on raising the temperature to 350° the substance did not dissolve after five hours. On cooling, the brown paste was skimmed off and dissolved in water.

On/

On acidifying, a blackish brown amorphous precipitate was obtained, which could not be purified. With the aid of the Lasseigne test it could be shown that the substance, though insoluble in acids, contains nitrogen.

Then, the rest of the potash fusion was dissolved in water, acidified and extracted with ether. On evaporation the smell of acetic acid was observed and a very small quantity of a solid was obtained. The low sublimation point (85°) and the insolubility of its calcium salt in water or acetic acid, suggested that it was oxalic acid.

Oxidation with potassium ferricyanide.

1 Gm. cevine was dissolved in 8 c.c. alcohol and 4 gm. potassium hydroxide in 8 c.c. water were added. A saturated cold solution of $K_3Fe(CN)_6$ was added gradually. The reaction was controlled by testing a drop of the reaction mixture with ferrous sulphate. The deep blue coloration indicating an excess of the potassium ferricyanide appeared after the addition of the amount equal to 9 atoms of oxygen. At the end of the reaction the/

the oxidation mixture had become practically a solid mass of yellow plates. These crystals, apparently the ferrocyanide of some organic base, were readily soluble in cold water and insoluble in alcohol. Efforts to isolate the organic matter from the complex were unsuccessful.

Oxidation with H_2SO_5

2 Gm. cevine were allowed to stay overnight with a mixture of 10 gm. K_2CO_3, and the K_2SO_4 formed was precipitated by the addition of alcohol. On evaporation of the alcoholic filtrate an amorphous compound was obtained. It was readily soluble in alcohol or in acetone. Attempts to crystallise it were unsuccessful.

Oxidation with Fehling's solution.

1 Gm. cevine was heated for 40 minutes with 50 c.c. of Fehling's solution. The oxidation mixture was acidified, filtered from cuprous oxide and the acid potassium tartrate and then extracted with ether. The ethereal solution was dried over Na_2SO_4 and evaporated. It was readily soluble in alcohol or acetone. Attempts to crystallise it/

it were not successful.

The mother liquor was made alkaline and extracted with ether. The ethereal solution gave practically no residue.

Oxidation with ammoniacal silver nitrate.

1 Gm. cevine was heated with an ammoniacal solution of 3 gm. $AgNO_3$ on the water bath. A silver mirror appeared very soon, but the reaction was finished only after many hours. The oxidation mixture was acidified with acetic acid and the silver precipitated with NaCl. The filtrate was evaporated in vacuo; the ammonium acetate was sublimed off at water bath temperature. The residue was dissolved in absolute alcohol, leaving $NaNO_3$ and NaCl undissolved. On evaporation of the alcohol an amorphous substance was obtained. It was readily soluble in water or alcohol, very little in ethyl acetate. However from ethyl acetate or from a mixture of ethyl acetate and alcohol it separated in an amorphous condition. Other attempts to crystallise it were also unsuccessful. It did not distil in a high vacuum.

Oxidation/

Oxidation with nitric acid.

2 Gm. cevine were refluxed with 25 c.c. concentrated nitric acid for 24 hours. On evaporating the nitric acid a pale yellow viscous residue was obtained. It was readily soluble in alcohol, acetone or dioxane, insoluble in ether or petroleum ether, but did not crystallise from these solvents or their mixtures. Efforts to crystallise it or to separate it into its constituents were not successful.

Oxidation with potassium permanganate.

3 Gm. cevine were dissolved in acetone (purified with $KMnO_4$) and finely powdered $KMnO_4$ was added in small portions with stirring. After the addition of 3.75 gm. (= 7 atoms oxygen) no more $KMnO_4$ was decolorised within four hours. The brown precipitate was filtered off and washed with more acetone. On evaporating the acetone solution practically no residue was obtained. The brown precipitate was then shaken several times with water and filtered, leaving the MnO_2 undissolved. Sulphur dioxide was passed through the aqueous solution/

solution to reduce traces of colloidal MnO_2, the SO_2 was blown off by a current of air and the solution acidified. It was extracted with ether, the ethereal extract dried over Na_2SO_4 and evaporated. A minute amount of a white amorphous powder was left. It was readily soluble in alcohol and acetone, but attempts to crystallise it were not successful. It did not sublime in a high vacuum. The mother liquor was then concentrated on the water bath and deposited soon a large amount of a black viscous mass. After decantation of the mother liquor, a brown-black amorphous compound was obtained. It was slightly soluble in water or acids, readily in alkalis or alcohol, On heating with a concentrated aqueous solution of potassium hydroxide, a coniine-like smell was observed and the vapours leaving the test tube turned red litmus paper blue.

Action of lead tetra-acetate.

2 Gm. cevine were dissolved in $CHCl_3$ and the solution was allowed to stand over Na_2SO_4 for several hours. The chloroform was then distilled off/

off and the residue dissolved in glacial acetic acid. 7.75 Gm. (= 5 mol.) of lead tetra-acetate were added gradually. Occasionally to a drop of the reaction mixture water was added to see whether it contains any unchanged lead tetra-acetate. The whole of the 5 mol. lead tetra-acetate disappeared, the solution became orange coloured, but the temperature did not rise. On evaporation in a vacuum a dark brown viscous substance was obtained. It was soluble in glacial acetic acid and aqueous alkalis, slightly soluble in hot alcohol, insoluble in $CHCl_3$, ether, water, or dilute mineral acids. By combusting on a platinum foil a yellow coloured ash was left behind, indicating that the substance was probably a lead complex.

Oxidation of cevine with hydrogen peroxide in alkaline solution.

To 1 gm. cevine, 20 c.c. of dilute (about 2 N) NaOH, 30 c.c. water and 25 c.c. H_2O_2 were added. The temperature rose to about 50°. Apparently the cevine did not dissolve. The following day a trace of PtO_2 was added and the substance gradually went into solution. At this stage the solution/

solution was extracted with ether, the ethereal solution dried over Na_2SO_4 and evaporated. The white amorphous residue was dissolved in a small quantity of ether, and petroleum ether (b.p. 40-60°) was added. The substance precipitated in micro-crystalline form. The yield was 130 mg. It was twice crystallised from much petroleum ether (80-100°) and had the m.p. 153°. On analysis it gave the following figures:

2.699 mg. subst. gave 6.12 mg. CO_2; 2.12 mg. H_2O;
C, 61.86%; H, 8.79%.

3.722 mg. subst. gave 8.51 mg. CO_2; 2.90 mg. H_2O;
C, 62.36%; H, 8.72%.

2.653 $p = 715$ mm., $t^\circ = 22$, $v = 0.089$;
N, 3.84%.

$C_{20}H_{33}O_6N$ requires C, 62.66%; H, 8.61%; N, 3.65%.

$C_{19}H_{31}O_6N$ C, 61.78%; H, 8.40%; N, 3.79%.

Determination of $C-CH_3$ by the method of Kuhn and Roth.

Mg. subst.	c.c. N/100 NaOH	No. of side chains
8.077	2.35	1.11
5.945	1.70	1.09

Thus the compound A has one side chain.

Determination of active hydrogen (pyridine, 19°).

Mg. subst.	c.c. CH_4	Active H for	
		$C_{19}H_{31}O_6N$	$C_{20}H_{33}O_6N$
3.382	0.99	4.86	5.05
3.046	0.92	4.95	5.17

In later experiments this compound will be referred to as compound A.

Compound A is micro-crystalline, m.p. 153°. It is readily soluble in alcohol, acetone, ether benzene and acids, moderately in water, slightly in petroleum ether. It turns gradually brown when heated with alkali. It contains no lactone group, five of its hydroxyls being alcoholic. The nitrogen is tertiary. Picrate, methiodide and benzoyl derivative could not be crystallised, the acetyl derivative seems to be soluble in water and could not be extracted with ether. On heating with concentrated acids the same deep red coloration appeared as with cevine.

United mother liquors were concentrated, acidified with hydrochloric acid, the precipitated sodium chloride filtered off and the solution extracted continuously with ether. The ether was dried/

dried over sodium sulphate and evaporated. A liquid residue was obtained, the smell of which suggested the presence of substances volatile in steam. The aqueous solution which was obtained on the steam distillation was made slightly alkaline, concentrated, acidified, and extracted with ether. The ethereal solution was dried over sodium sulphate and evaporated. A few drops of a liquid were obtained which had a strong smell of acetic acid. The substance was distilled and neutralised exactly with aqueous sodium carbonate; then alcohol and paraphenylphenacyl bromide were added and the mixture refluxed for two hours. Water was added and the paraphenylphenacyl ester precipitated. It was filtered, washed, dried and crystallised from alcohol. No m.p. depression with the paraphenylphenacyl ester of acetic acid, m.p. 108°, was observed.

A substance, not volatile in steam, was extracted from the aqueous solution with ether. On evaporating the dry ethereal extract an orange coloured viscous compound was obtained in a fair quantity. It could not be crystallised. The paraphenylphenacyl ester was prepared which separated/

separated from alcohol in an amorphous form. Using the Lasseigne test, the presence of nitrogen could be shown. On heating with strong alkali a base distilled with the vapours, which turned red litmus paper blue.

The mother liquor of the oxidation mixture was then made alkaline again and evaporated to dryness. The brown residue was continuously extracted with acetone. In the thimble a white crystalline compound was left. It was dissolved in much hot methyl alcohol and filtered from inorganic impurities. On cooling, the substance separated in white needles, m.p. 273°. Two further crystallisations raised the m.p. to 278°.

The analysis gave the following results:

3.543 mg. subst. 2.66 mg. H_2O; 8.05 mg. CO_2;
8.40% H; 61.97% C.

3.743 mg. subst. 2.82 mg. H_2O; 8.47 mg. CO_2;
8.43% H; 61.71% C.

$C_{27}H_{43}O_9N$ requires C, 61.71%; H, 8.19%.

The substance gave no m.p. depression with cevine oxide, m.p. 278°, prepared from cevine and H_2O_2 by the/

the method of Freund (loc. cit.).

Determination of active hydrogens (Pyridin, 18°)
5.35 mg. subst. c.c. CH_4, 1.38.
Calc. for $C_{27}H_{43}O_9N$ 6.03 active hydrogens

From this follows that six active hydrogen atoms are present in cevine oxide.

On evaporating the acetone extract (obtained from the oxidation mixture) a dark brown viscous compound was obtained. It could not be crystallised.

Action of ferric chloride on cevine.

To 1 gm. cevine, dissolved in 40 c.c. water containing a few drops of hydrochloric acid, 4.7 c.c. of a 20% ferric chloride solution (= 1 atom oxygen) were added in the cold. A drop of the reaction mixture was tested with an aqueous solution of potassium thiocyanate from time to time, and was always found to contain ferric ion. On heating on the water bath the solution became turbid and gradually deposited a pale brown substance. On testing the reaction mixture again with potassium thiocyanate the presence of ferric ion/

ion could still be shown. The brown precipitate was insoluble in acids, and since no oxidation occurred it seems to be an iron complex.

Action of phosphorus pentoxide on cevine.

1 Gm. cevine was dissolved in toluene and the solution was dried over Na_2SO_4. P_2O_5 was added and the mixture heated on the water bath. A red amorphous compound separated. The toluene was decanted off and evaporated to dryness. It contained practically no residue. The red amorphous substance was dissolved in water, the solution made alkaline, evaporated to dryness and the residue extracted with ether. (The formation of an emulsion made the extraction of the alkaline solution impossible). On evaporating the ethereal solution, a green amorphous base was obtained in fair quantity. It was readily soluble in alcohol or acetone, and slightly soluble in ether. Attempts to crystallise the substance were unsuccessful.

Methylation/

Methylation of the compound A.

100 Mg. of the base were refluxed with 61 mg. Ag_2O (= 5 mol) and 1 c.c. methyl iodide for 3 hours. The methyl iodide was evaporated and the residue extracted with alcohol. Since the substance did not crystallise from the concentrated solution, the alcohol was evaporated and a white amorphous substance was obtained. It was soluble in alcohol or acetone, insoluble in water and ether. Attempts to crystallise it were not successful.

200 Mg. of the compound A were dissolved in toluene (dried over P_2O_5) and 36 mg. K_2CO_3 and 0.49 c.c. $(CH_3)_2SO_4$ were added. A red amorphous compound precipitated immediately. Attempts to crystallise it were not successful.

Action of silver oxide in ether on the compound A.

100 Mg. of the base were refluxed with dry Ag_2O in absolute ether. At the end of the reaction the starting material was recovered unchanged.

Action/

Action of phosphorus pentoxide on the compound A.

100 Mg. of the base were dissolved in toluene and after the addition of P_2O_5 the mixture was heated on the water bath. The toluene was decanted off and evaporated to dryness. No residue was left. The red precipitate was dissolved in water, made alkaline and extracted with ether. The ethereal extract was dried over Na_2SO_4 and evaporated. A green amorphous base was obtained. Attempts to crystallise it were not successful.

Action of lead tetra-acetate on the compound A.

100 mg. of the base were dissolved in benzene and 113 mg. lead tetra-acetate (= one atom oxygen) were added. Immediately a white amorphous complex precipitated. The benzene solution was filtered off and evaporated to dryness. It contained practically no residue.

Action of p-nitrophenylhydrazine on the compound A.

100 Mg. of the base were refluxed in 50% acetic acid solution with 40 mg. p-nitrophenylhydrazine for one hour. On diluting the clear solution/

solution with water, no substance separated. The solution was made alkaline with ammonia and extracted with ether. The ether was dried over Na_2SO_4 and evaporated. A small amount of a yellow crystalline compound was obtained, but this proved to be the acetyl derivative of p-nitrophenylhydrazine.

Zinc dust distillation of cevadine.

Approximately 0.3 gm. cevadine was mixed with an excess of zinc dust and heated in glass tubes in a hydrogen stream. At 260° a substance distilled which crystallised almost immediately. The tubes were opened, the crystalline substance (collected from six distillations) was pressed on a porous plate and redistilled. The distillate solidified soon to a mass of colourless crystals, m.p. 42°. The crystals were mixed with tiglic acid and the mixture melted before it could be transferred to the m.p. tube.

On titration the following result was obtained.

7.8 mg. substance were neutralised by 4.73 c.c. 0.0173 N NaOH.

Equivalent weight = 95; required for angelic acid 90.

7.8 Mg. substance were heated in a 30% aqueous

NaOH/

NaOH solution on a water bath overnight. The solution was diluted with water, acidified and extracted with ether. The ethereal solution was dried over Na_2SO_4 and evaporated. The residue was distilled. On cooling the distillate, small crystals appeared mixed with an oily substance. These were collected with the aid of a spatula and pressed to a porous plate. The yield was just sufficient for one m.p. It was 56-57°. A crystal of tiglic acid was added and the mixture heated above the m.p. and cooled again. The mixture crystallised immediately on cooling and melted at 63-64°.

Thus the substance which was different from tiglic acid before, became identical with tiglic acid after heating with alkali, and was therefore obviously angelic acid.