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TO: [illegible]
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ATTN:

BUILDING 12 ROOM NO. 30

TITLE

Translation of Japanese Patent #[illegible]/
Manufacturing of Ten Milligram Content of
Ergot by Artificial Culture (Japan)

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REMARKS

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Japanese Government

The Detail of the Patent and Invention #178336

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"The manufacturing of new effective content of ergot by artificial culture."

I) The abstract of the nature and object of the invention.

The purpose of this invention is to prepare the very effective new material which possesses extremely powerful action to contract the uterus from the artificial culture of ergot bacteria which produces new ergot alkaloid, Agroclavin. The general procedures are as follows: first to culture the ergot bacteria which produces alkaloid, Agroclavin, on the bases such as malt juice, Koji juice or other synthetic bases; secondly the extraction of the ergot alkaloid from bacteria colony or cultural base (in the latter case extraction is carried out from itself or extract from the alumina which previously adsorbed the alkaloid) by organic solvents, in alkaline solutions such as ether, chloroform, benzene, methanol, acetone, toluene, pyridine, carbon disulfide or carbon tetrachloride, etc. are carried out.

1) The extract is decolorized by the activated charcoal, etc. and then condensed to give crude or
2) Re-extracted by organic or inorganic acids such as hydrochloric acid, sulfuric acid or tartaric acid etc. To the acid solution, ammonia, etc. (alkaline material) was added to precipitate Agroclavin from impurity.

II) Detailed explanation of the invention.

This invention is the addendum to the original invention in which the Ergot bacteria was cultured in the malt juice and other artificial cultural bases and the cultural base was directly extracted with organic solvent such as ether, chloroform, benzene, etc. which are not miscible with water or adsorbed by adsorbent such as alumina to get active component of ergot, but after the further research, the ergot bacteria, which produces new alkaloid--- Agroclavin, was cultured in the malt juice, Koji juice or other

artificial cultural base, then from this there was obtained the crystalline material which possessed extremely powerful action of contraction of uterus and the properties of this material do not correspond to the known ergot alkaloids, so this was named Agroclavin. Its molecular formula is $C_{26}H_{28}O_2$, m.p. 198-200° C $(\alpha)_D^{20}$ = -142° (chloroform) and the maxima absorption spectra has the peak at 2325 A°.

The recrystallized material from ether was the colorless monoclinic crystals. This material gives ppt. with phosphorus tungstan acid or Mayer reagent. Its sol. in inorganic or organic acids and their solution did not show $FeCl_3$ test. It gives blue coloration to the sulfuric acid solution of vanillin, p-dimethylamino benzaldehyde, etc. It is very difficultly sol. in CH_3OH, H_2O, NH_3, ether, CCl_4 and readily sol. in ethyl acetate, CS_2. The toluene solution, etc. shows slight alkalinity. 1:10,000,000 dilution gives marked pharmacological action upon the isolated uterus of rabbit or guinea pigs.

The extraction of Agroclavin from the bacterial colony or cultural base which was cultured on cultural bases such as malt juice or koji juice or other synthetic bases was carried out as follows:

1) Bacterial colony was separated from cultural solution by the filtration and washed and pulverized at low temp. and removed the fats by pet. ether or ligroin, etc. And the powder was made basic and extracted with ether, NH_3, Acetone, CH_3OH, $HCCl_3$, pyridine, CS_2, CCl_4, etc. to extract Agroclavin. When necessary the extract was decolorized by charcoal and condensed slowly to give the crystal or crystalline powder of Agroclavin. And the solution of Agroclavin in organic solvent or its condensed solution was shaken with the acid such as HCl, H_2SO_4, boric acid, etc. and then the acid solution was made alkaline with ammonia or other alkaline material and then again it was extracted with the organic solvent. When this operation was repeated it was harmless to change the solvent during the extraction. When the repeated re-extraction solution was condensed, the crystal or crystalline powder of Agroclavin separated out.

When the acid solution, which was the acid extract from the organic solvent containing Agroclavin, was made alkaline, Agroclavin, free from the impurity, precipitated at once according to the degree of the condensation of the solution and respecting to the amount of the acid used.

The ppt. was filtered and washed and dissolved in the organic solvent, which was dry when necessary, and organic solution was condensed to give the crystal or the powder of Agroclavin.

2) From the culture-base:

The culture-base which was filtered from the bacteria-colony, with or without condensing the volume, was extracted with ether or others which are not miscible with water or adsorbed on the alumina etc. Under alkaline media it was extracted with the organic solvent and the solution was dried and decolorized with charcoal thoroughly, then condensed to give the crystal or the powder of Agroclavin.

Or it was shaken with the acid and the acid solution was made alkaline with ammonia or other alkaline material to ppt. Agroclavin free from the impurity. The ppt. was filtered and washed and then dissolved in the organic solvent such as ether, benzene, acetone or methanol and the solution was dried when necessary and condensed to give the crystal or the powder of Agroclavin.

The ppt., crystalline powder or the crystal of Agroclavin obtained from the bacteria-colony or culture-base as mentioned before was dissolved in the water-miscible organic solvent such as methanol or acetone, etc. for the recrystallization and the solution was filtered and condensed if necessary.

The crystal was obtained when water was added to its solution

Experimental procedure was as follows:

Experimental:

1. Ergot bacteria, which produces Agroclavin, was placed in the malt juice (pH 5.4) and it was cultured at 28° C for 37 days. Then the bacteria-colony was filtered from the culture-solution and pressed and dried at low temperature. The dried colony was pulverized and treated with pet. ether to remove fat. This powder was wet with ammonia water to make it alkaline and extracted with ether to dissolve the containing alkaloid in ether. Etherial solution was dehydrated and treated with charcoal. The condensed etherial solution was placed in the desiccator to separate the powder of Agroclavin from the solution.

The collected crystalline powder was dissolved again in ether and the solution was dehydrated and decolorized and then condensed to give the crystalline Agroclavin (0.4 g. of crystal per every 100 g. of defatted powder).

2. 5.0% of sucrose, 0.1% of potassium hydrogen phosphate, 0.03% magnesium sulfate, 0.8% of ammonium succinate in water was made pH 5.2 by addition of succinic acid.

To this artificial culture-base was placed the sample ergot bacteria, which produces Agroclavin, and it was cultured at 28° for 40 days. Then the colony was filtered from the base and pressed and dried at low temperature to obtain the powder, which then defatted and wet with ammonia water and then extracted with benzene. The condensed benzene solution was extracted with small amount of 1 % tartaric acid solution repeatedly. The combined acid solution was made alkaline by dropwise addition of ammonia-water to ppt. Agroclavin free from the impurity. The filtered ppt. was washed with dil. ammonia solution and then dissolved in ether. The dehydrated etherial solution was condensed to give crystalline Agroclavin (0.5 g. from every 100 g. of bacteria-colony). The mother liquor, after ppt.ating the alkaloid, was extracted with ether and the etherial solution was dehydrated and decolorized and condensed to give crystalline powder of Agroclavin.

3. The aqueous solution consists of 5% of mannitol, 0.1% of potassium hydrogen phosphate, 0.03% of mg. sulfate, and 1.0% of asparagine and was made pH 6.4 by sodium hydroxide solution. To this artificial culture-base was put the sample bacteria, which produces Agroclavin, and it was cultured at 28° C for 17 days and then at 24° C for 15 days additionally.

On the 33rd day the cultured-base was filtered from the bacteria colony and the solution was treated with alumina (1 g. of Al_2O_3 per 3.0 mg. of alkaloid in the solution) to adsorb the alkaloid almost completely and then the alumina was extracted with ether under the ammonia-alkalinity. The condensed etherial solution was repeatedly extracted with 3% succinic acid solution. To the acid solution was added ammonia solution dropwise to make it alkaline to free and ppt. the Agroclavin from the impurity. The ppt. was filtered and washed and then dissolved in acetone. Insol. material was filtered off and the acetone solution was then condensed and then diluted with dropwise addition of water. And when the crystal of Agroclavin began to separate the solution was placed in the cold room to obtain the crystalline Agroclavin (0.2 g. of crystal per 1000 cc of the culture-base).

The Relations to this Invention

This invention is the addendum to the pat. 169,919 and in the original invention the ergot bacteria was cultured in malt juice, Koji juice or other artificial culture-bases and the base was extracted directly with the organic solvent such as ether, $CHCl_3$ or benzene, etc. or it was treated with alumina to adsorb the

...ing component of ergot. This invention was the improvement and expansion of the original invention. All here the ergot bacteria producing new alkaloid Agroclavin, was cultured in the malt juice, Koji juice or the artificial base. The active component of ergot from the bacterial-colony or the culture base, which contains the Agroclavin, was extracted with the organic solvent, under the alkalline media, such as ether, $CHCl_3$, C_6H_6, methanol, acetone, C_5H_5N, pyridine, CS_2, CCl_4 etc. The solution was 1) decolorized with bone-coal or active charcoal, etc. and condensed or 2) dissolving the component in the various acids such as HCl, H_2SO_4, tartaric acid, etc. To the acid solution was added ammonia solution or other alkalline substance to free and ppt. the Agroclavin from the impurity to obtain the crystalline new pd. Agroclavin which has very powerful action and contract uterus.

The domain covered by this patent

As stated previously, the ergot bacteria, which produces new alkaloid Agroclavin, was cultured on the malt juice, Koji juice or other artificial culture base. The bacteria colony or the culture-base (in the latter case, extraction was done directly from the culture solution or the component was adsorbed on the alumina), under alkalline condition, was extracted with the organic solvent such as ether, $CHCl_3$, C_6H_6, NaOH, acetone, C_5H_5N, pyridine, CS_2, CCl_4, etc. The solution was 1) decolorized with charcoal and then condensed or 2) extracted into the acid such as HCl, H_2SO_4 or tartaric acid, etc. To the acid solution was added eq. ammonia solution or other alkalline solution to free and ppt. Agroclavin from the impurity.

The patent covers the above-mentioned method to obtain the new active component, Agroclavin, of ergot.