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PHYTOCHEMICAL NOTES.*[1]

No. 110. DIGITONIN AND PHYTOSTEROL FROM THE SEED OF DIGITALIS PURPUREA.

BY OLE GISVOLD.

A kilo of seed, not comminuted, was extracted with a liter of alcohol in a continuous extraction apparatus for 8 hours and the extract tested for digitonin with cholesterol. The extraction was repeated with a like amount of solvent for 10 hours and a third time for 12 hours. The third extract gave but a slight precipitate with cholesterol.

The same process was repeated with comminuted seed. So far as the digitonin was concerned, the results appeared to be about the same. However, the comminuted seed gave up more of its fatty oil. These results might have been anticipated, since the digitonin is located in the seed coating, whereas the fatty oil is located in the cotyledons.

The balance of the available seed, 25 pounds, all of which had been raised in the Garden of the Pharmaceutical Experiment Station under the direction of Prof. W. O. Richtmann, was ground and extracted in the Lloyd extractor.

The first alcoholic concentrates removed had separated a considerable amount of fat with which was mixed a solid substance that was removed by force filter. Hence the alcoholic concentrate yielded two products, *viz.:*

1. The solid that remained in the filter and which was shown to be a digitonide, and digitonin.

2. The filtrate which consisted of the alcoholic extract plus dissolved and separated fat.

1. Purification and identification of the digitonide. The greenish material which had remained in the filter was several times suspended in hot petroleum ether to remove fat which was added to the other petroleum-ether extracts. After that it was suspended several times in hot alcohol to remove any alcohol-soluble material. The substance thus purified was designated "A;" the combined alcoholic filtrates were designated "B."

(A) The purified and pulverized solid weighed 18.5 Gm. It was suspected to be digitonide, since the digitonin from the seed coats and the sterol from the cotyledons would naturally be brought together in the alcoholic extract. In so far as the two dissolved substances met within the tissue of the seed, the insoluble digitonide resulting should be found in the extracted marc. In so far as the two substances reacted in the percolate, they would precipitate each other.

* From the laboratory of Edward Kremers.

[1] Scientific Section, A. PH. A., Madison meeting, 1933.

In order to test this hypothesis, the substance was treated with boiling xylene in the usual manner (1). The extract precipitated digitonin (2) and gave good positive color reactions with both the Liebermann-Burchard (3), and the Salkowski (4) tests. Hence it was pronounced a sterol. It melted at 137° to 138° (5) and yielded an acetate which melted at 130° to 131°. No change in the melting point of the alcohol could be observed when mixed with the sterol obtained from stramonium seed. According to Windaus' test, it contained no stigmasterol (6). Culter reports that the sterol isolated by him (7) melted between 93° and 105°, which very fact proclaimed it as impure. Examination revealed his impure sterol to contain hydrocarbons, too minute in quantity to be identified. When purified by the removal of this hydrocarbon it had the same melting point as that found by the writer.

The residue in the extraction capsule was digitonin. It had the capacity to precipitate sterols, also gave the Keller color reaction (10).

(B) The alcoholic filtrate "B" upon cooling and standing yielded a light greenish precipitate. Its solution in 85 per cent alcohol, after boiling with animal charcoal, yielded a colorless filtrate which upon cooling deposited a white crystalline material. Inasmuch as it had the capacity to precipitate cholesterol, it was assumed to be digitonin. It likewise gave a positive Keller color reaction (11).

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The original alcoholic concentrate, from which the substances described above had been removed by filtration, was defatted in a manner described and illustrated in another paper (8).

I. The petroleum-ether residue, consisting, no doubt, for the most part of fatty oil, was set aside. Culter not long ago reported on the constituents of the fatty oil of digitalis seeds (7). If deemed desirable, the fat may be reëxamined later.

II. To the defatted alcoholic concentrate, heated almost to the boiling point, successive portions of cholesterol (9) dissolved in 95 per cent alcohol were added until no further precipitate occurred. The combined precipitates were suspended several times in hot alcohol to remove alcohol-soluble impurities. The air-dried and powdered material weighed 275 Gm.

The digitonide thus obtained was decomposed in 15-Gm. portions, with boiling xylene in the usual manner (1). The mixed, impure digitonin was extracted four times with hot 95 per cent alcohol. To the first extraction, while warm, ether was added until the cloudiness first produced just disappeared. Upon cooling, the digitonin crystallized out. The subsequent extractions were concentrated before the ether was added to the warm concentrate. From the mother liquids additional digitonin was obtained by cooling in a salt and ice bath. Subsequently the combined mother liquids were evaporated under vacuum to a syrupy consistence and the residue dissolved in the smallest amount of hot 95 per cent alcohol. The digitonin thus obtained was more highly colored than the products previously separated.

The combined residues of the xylene-treated material in the capsules, after exhaustion with alcohol to remove the liberated digitonin weighed 94 Gm., apparently undecomposed digitonide. This was again treated with xylene but again a residue of 40 Gm. of apparently undecomposed digitonide resulted. A third treatment still yielded a small residue which was set aside temporarily.

The total amount of crystalline, sterol-precipitating material, *i. e.*, digitonin, thus obtained was about 155 Gm. corresponding to 1.16 per cent of the seed. It should be remembered, however, that a small amount of precipitated digitonide was not resolved into its components, also that some of the digitonin had been separated from the alcoholic extract as insoluble digitonide, having been formed during the process of extraction. As yet it has not been ascertained whether the digitonide, removed as previously described, constitutes all of the digitonide formed during the extraction. As previously pointed out it seems reasonable to assume that digitonide may be precipitated in the tissue during the process of extraction, hence may be lost in the marc.

That the 155 Gm. of material, separated as described, consisted of digitonin was demonstrated not only by its method of preparation which is sufficiently specific to exclude other compounds, but by its m. p. 227° to 240° (10) (not very conclusive it is true), its ability to precipitate sterols (quite conclusive), also by a positive Keller color reaction (11).

The defatted alcoholic concentrate from which the digitonin had been precipitated with cholesterol was set aside to be investigated at a later date, if deemed desirable.

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(7) *Am. J. Pharm.*, 102 (1930), 545.
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(9) The cholesterol used was obtained from human gall-stones by extraction of the crushed gall-stones with ether and subsequent crystallization from alcohol.
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TWO SPECIES OF THE GENUS LEDUM.*

BY RUSSELL A. CAIN AND E. V. LYNN.

Several years ago we reported (1) on the volatile oil of *Ledum grœnlandicum* Oeder., which grows abundantly in the numerous bogs of Washington. We have now collected specimens of this plant from a dry bog near Seattle and examined them more carefully. We have also gathered samples of *L. columbianum* Piper, which is found along the coast of Oregon and Washington. It is differentiated from *L. glandulosum* by some and by others is considered identical. It is distinguished from *L. grœnlandicum* by being glabrous and by its larger, not revolute-margined leaves, which are not tomentose. Like the other species it is reputed to be poisonous to stock, but no one has hitherto investigated it scientifically.

* Scientific Section, A. PH. A., Madison meeting, 1933.