product was freed from the unchanged isatin derivative by repeated extraction with boiling alcohol.

Hg—Found 26.0%. Calc. for $C_{24}H_{19}NO_5Br_2Hg$ 26.4%.

3,3'-Dinitro 5,5'-Dimethyl Diphenol Isatin.—24 Gm. di-*o*-cresol isatin (5) was dissolved in 25 cc. glacial acetic acid and treated with 10 cc. HNO_3 (Sp. Gr. 1.4). After warming on the water-bath for 15 minutes the product was precipitated by adding water and recrystallized from alcohol. Yellow micro-crystalline powder m. p. 238–240° C.

N—Found 9.68%. Calc. for $C_{22}H_{19}N_3O_7$ 9.65%.

Diacetoxy Mercuri 3,3'-Dinitro-5,5'-Dimethyl-Diphenyl Isatin.—The nitro compound when mercurated in alcohol by the general method gave a partially trimercurated product, as in the case of the other nitro derivatives.

Hg—Found 44.7%. Calc. for $C_{24}H_{18}N_3O_9Hg_2$ 43.3%.

SUMMARY.

A number of new derivatives of diphenol isatin have been prepared. The mercury derivatives of these compounds have been prepared and their bactericidal properties investigated.

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THE VALUE OF SENECIO IN MEDICINE.*,[1]

BY EDGAR A. KELLY AND E. V. LYNN.

In a preliminary report two years ago (3) we recorded a partial examination of senecio. Since then we have completed the study and extended it to include the pharmacology.

CHEMICAL.

The material employed was obtained in the open market and was entirely within the official requirements, but we have no knowledge of place or time of gathering.

Proximate analysis of various samples gave results as follows: moisture 6.49 to 10.92 per cent; ash 8.33 to 11.85 per cent; starch, by diastase 7.85, by acid hydrolysis 10.20 per cent; tannin 6.14 per cent.

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For the purpose of complete examination, 13 Kg. of the ground drug was exhausted with hot alcohol. After removal of the greater part of the solvent, there was obtained 2.67 Kg. of a dark, viscid mass.

Volatile Oil.—By steam distillation of this extract there was produced 13.6 Gm., or 0.1 per cent, of an essential oil which was acid to litmus and possessed the characteristic, aromatic and spicy odor of the drug. It was light amber in color when first obtained but darkened on standing to a reddish brown color, thus exhibiting the presence of unstable compounds. The oil thus changed had the following constants: d_{25} 1.0072; n_D^{20} 1.4942; $[\alpha]_D$ + 27.91°; acid number 52.47; ester value 89.63. It gave no test for nitrogen or sulphur when submitted to the sodium-fusion method; the former report of finding sulphur was probably due to contaminated steam. The previous refraction (1.4511) was obtained with only a few drops of oil.

After rectification by steam, the oil had the following constants: d_{25} 0.9862; n_D^{20} 1.4921; $[\alpha]_D$ + 40.74°; acid number 25.33; ester value 60.47. These indicated unsaturated or aromatic compounds with comparatively large amounts of free acids and some esters.

Aqueous Residue.—The residual, dark brown, aqueous liquid was separated from the resins and evaporated to a convenient volume under reduced pressure. Repeated extraction with ether gave 25 Gm. of semi-solid, from which ammonium carbonate withdrew 16.4 Gm. (0.12 per cent) of oil representing free, non-volatile acids. Sodium carbonate solution extracted 1.15 Gm. (0.01 per cent), more than half of which crystallized in long needles melting at 129–130° C. and which was probably a lactone. It was soluble in benzene, ether, chloroform or hot water, but insoluble in petroleum ether or cold water, and was acid to litmus. From the original ethereal extract sodium hydroxide solution removed 0.41 Gm. (0.003 per cent) of phenols.

From the aqueous concentrate amyl alcohol extracted 32 Gm. (0.25 per cent) of a syrupy material. Most of this remained liquid even after standing for several months, but there separated 0.6 Gm. of a brown, amorphous powder which was apparently an anthraquinone derivative.

In the remaining aqueous product, which represented chiefly sugars, nothing else beside sugars was found. At no time was there any indication of glucosides and no characteristics of a saponin.

The Resins.—The solid portion from distillation amounted to 950 Gm. or about 7 per cent of the original drug. Of this black tarry mass, representing the fixed oil, phytosterol, resin, wax, etc., 18 per cent was removed by petroleum ether and subsequently 15 per cent by ether, both extracts being dark green and unctuous. Chloroform extracted from the residue 14 per cent of a black, brittle solid and alcohol then removed 9 per cent of black semi-solid, these probably being chiefly wax and resin. Nearly half of the original remained as a black mass.

Alkaloid.—In our previous report the presence of alkaloid was indicated. In order to determine this more carefully a large quantity of drug was exhausted with alcohol and the latter was largely removed by distillation. The residue was extracted with 2 per cent hydrochloric acid, producing a brown solution which gave copious precipitates with Wagner's or Mayer's reagents and with phosphotung-

stic acid, phosphomolybdic acid or picric acid, but none with tannic acid, Dragendorff's or Marme's reagents, platinic chloride or gold chloride.

The acid solution was made alkaline and extracted with a mixture of three parts ether and one part chloroform. The dried extracts were combined and evaporated to dryness, leaving a green, mobile oil corresponding to 0.006 per cent of the dried plant. It was acid to litmus and possessed a bitter and burning taste.

Weighed portions of the residue were tested for nitrogen by the sodium-fusion method. Amounts representing as much as 600 Gm. of the crude drug failed to give a positive test. Since, in our hands, as little as 4 mg. of strychnine will give the characteristic blue, and assuming that the proportion of nitrogen is about the same, we conclude that alkaloids are not present in senecio in amounts greater than 1:150,000.

At the time this work was being conducted, Manske (4) was also testing senecio for the presence of alkaloid. His results, which did not include a test for nitrogen, led him to draw doubtful conclusions.

Inulin.—Precipitated calcium carbonate was mixed with 600 Gm. of the crude drug in order to neutralize plant acids and the mixture was introduced into an equal volume of boiling water and heated on the steam-bath. After about four hours it was strained through muslin and expressed. These processes were repeated three times, when the liquid was filtered until clear and the tannins, colors, etc., were precipitated with neutral lead acetate. Basic lead acetate produced no more precipitate. After removing the lead with sodium phosphate the filtrate was reduced to one-third its volume by evaporation under reduced pressure.

Addition of an equal volume of alcohol caused precipitation of a white amorphous substance which was filtered and washed with alcohol and ether. The tasteless substance, resembling starch in appearance, was insoluble in cold water and organic substances but, unlike starch, gave a clear solution in hot water. After purification its solution was neutral to litmus, produced no change with iodine and did not reduce Fehling's solution. After hydrolysis by boiling with dilute hydrochloric acid for one minute, however, it produced an immediate reduction of hot Fehling's solution. A hydrolyzed solution gave a deep red color and a red precipitate when boiled with resorcinol in hydrochloric acid, thus proving the hydrolytic product to be a ketone sugar. The specific rotation was $-38.6°$, of pure inulin $-39.5°$, of soluble starch $+169.1°$. The hydrolytic product had a specific rotation of $-95.7°$, comparable to the $-98.8°$ of levulose. The original substance is thus proved to be inulin, which is present to the extent of about 7.85 per cent.

While inulin is quite prevalent in plants of the composite family, this is the first instance we can find that it has been found in a senecio.

The liquid from which the inulin had been precipitated was evaporated to a small volume under reduced pressure and was then allowed to stand for several weeks, being subject to some evaporation in the meantime. Nothing crystalline was thus obtained and other properties of the residue would seem to indicate that glucosides are either absent or present only in very small quantities.

PHARMACOLOGICAL.

Attempts to isolate and identify compounds to which the reputed value could be attributed having failed, attention was next turned to the pharmacology.

This involved determination of toxicity, action on the excised uterus and on the uterus in a living animal.

Toxicity.—The animals used were two fully grown, healthy, white rats, one male and one female. The fluidextract, administered orally by means of a medicine dropper, was given in increasing doses at intervals of one or two days in order that there would be no accumulation. No effects were noted at any time. Since the largest dose corresponded to 100 times the average for man, the fluidextract can be considered non-toxic to rats.

Weighed quantities of the crude drug were mixed with glucose, rolled into pills and administered to a rabbit weighing 2406 Gm. Three doses were given at intervals of two days. The first, 4 Gm. of senecio, corresponded to 0.17 per cent of the body weight, the second of 12 Gm. to 0.5 per cent and the third of 24 Gm. to 1.0 per cent. In order to get the same amount of drug per Kg., the average adult person would need to ingest 700 Gm. None of these enormous doses produced any outwardly evident effects and it can be concluded that senecio is entirely non-toxic.

Isolated Uterine Strip.—The only previous investigation of this nature on senecio was made by Pilcher in 1916 (2), who finally concluded that the fluidextract gives no constant action on the excised uterus of guinea pigs. In high concentration, however, there was an indication that the drug is depressant.

The animals used were adult, non-pregnant rabbits and cats. The rabbit was killed by a blow on the head, the vessels in the neck were cut and the animal was rapidly bled. The abdomen was opened and the entire uterus, including the ovaries and a part of the vagina, was removed to a beaker containing Ringer-Dale solution. The cats were anesthetized by ether instead of being killed before bleeding.

On a warm, moistened tile, one horn of the uterus was cut longitudinally with sharp scissors and, with the muscle laid flat, a segment was made 1.5 cm. long and 0.5 cm. wide. Holes were pierced in each end to receive pieces of silk thread which were tied securely, one being attached to a muscle lever, the other to a writing lever. The uterine strip, with its attached muscle lever, was then placed in a Harvard warmer containing 50 cc. of Ringer-Dale solution, which was well oxygenated and maintained at about 38° C. by means of an outer water-bath. The jacket of the latter was a galvanized pail in the bottom of which was a hole to receive a rubber stopper. A short glass tube was passed through this stopper and connected by means of a rubber tube to the muscle chamber. The outer end of the glass tube was attached to a T-tube, one arm of which served as a washout for the chamber, the other being connected to a second T-tube. The second arm of the latter led to the oxygen tank and the third to a reservoir of fresh saline kept at 38° C. Thus, by regulating various screw clamps, the solution surrounding the muscle was kept well oxygenated and could be washed out and renewed with fresh, warm saline in a fraction of a minute. The thread from the upper end of the uterine strip was attached to the short arm of a counterbalanced lever which recorded contractions by an upstroke of the writing point on a slowly moving kymograph drum.

After obtaining a satisfactory normal tracing, free from spontaneous and erratic movements, the drug was added at the top of the chamber and away from the

strip by means of a graduated capillary pipette. It was allowed to act for ten minutes or longer. The preparation used was the official fluidextract prepared by Eli Lilly & Co. containing 55 per cent of alcohol, control experiments being run with the evaporated fluidextract, with 55 per cent alcohol and with fluidextract of ergot (Lilly). The results with the evaporated preparation did not differ materially from those of the official one, while the tests with alcohol showed that this ingredient has little effect on the uterine strip. The fluidextract of ergot caused the usual pronounced stimulation in all controls.

The concentrations used were much higher than those which could be produced in the body. If we assume that the average dose, 4 cc., be completely and rapidly absorbed, there would be about 1 : 17000 in the blood of an adult man. In our experiments the concentrations employed were 1 : 1000 and 1 : 500.

Out of a total of ten experiments using a dilution of 1 : 1000, rate and amplitude were increased in one and remained constant in nine. The tonus was raised in two and was unchanged in eight. In the three cases the rises were very small. Out of nineteen experiments using the dilution 1 : 500, the rate was raised in five and lowered in two and the amplitude and tonus were increased in five and decreased in three. Each change, however, was relatively insignificant, being in no case more than a small fraction of the alterations produced by ergot. We may justifiably conclude that even very high concentrations of senecio have no action on the excised uterus.

Intact Uterus.—Since it is impossible to simulate outside the body the conditions which are so rigidly controlled inside the organism, the preceding experiments on excised tissue do not represent the normal effect of the drug. The mode of administration and absorption of the drug, temperature control and oxygen supply and consumption are necessarily greatly changed when the organ is removed. The injury involved in severing the organ from the body and from the nervous mechanism supplying it is a factor not met in studying the activity in the intact animal.

The method of investigation was that of Barbour (1), using fully grown, non-pregnant cats. About one hour was allowed to elapse after the operation had been completed and the apparatus had been adjusted, in order to afford sufficient time for the establishment of regular uterine movements, which were in all cases small. After obtaining a satisfactory record of the normal contractions, the fluidextract was injected in doses of 0.5 cc. through the cannula in the left jugular vein. A control injection of 0.5 cc. of fluidextract of ergot was made at the end of each experiment. In all, five experiments were performed using as many animals. In each fluidextract of senecio failed to change the character of the uterine movements in any respect, while fluidextract of ergot produced the characteristic stimulating action.

CONCLUSIONS.

Although senecio has been used in medicine for many years, chiefly in uterine disorders, we find no published evidence of any value. The drug is official probably because of some considerable usage, but certainly this cannot be because of scientific proof. Since immense doses of the drug give no demonstrable effects on animals, since local application to the uterus gives no action on the rate, amplitude or tone

of the uterine muscle, we are forced to the conclusion that the drug is valueless in the conditions for which it is prescribed. We offer the suggestion that it be eliminated from our materia medica. As long as certain physicians prescribe it, deletion from the formulary may not be deemed advisable, but it would seem logical to urge strongly abandonment of any administration.

SUMMARY.

A proximate analysis of senecio was made, including moisture, ash, tannins, resins, etc. There was found 0.1 per cent of volatile oil whose constants are given, also about 8 per cent of inulin and no starch. No evidence could be found for the presence of alkaloids or glucosides.

Even with enormous doses, senecio caused no untoward effects in rats or rabbits. Numerous experiments on isolated uterine muscle and on normal uterine movements in intact animals clearly demonstrated the absence of any effect on the tone, rate or amplitude of this muscle.

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THE ASSAY OF CAFFEINE SODIO-SALICYLATE AND ELIXIR OF SODIUM SALICYLATE.

BY EDWARD M. HOSHALL, DONALD C. GROVE AND GLENN L. JENKINS.

CAFFEINE SODIO-SALICYLATE.

This preparation has been recommended for admission into the National Formulary VI. A method of assay is proposed.

Assay for Caffeine.—Transfer 2.0 Gm. of caffeine sodio-salicylate, previously dried to constant weight at 80° C., and accurately weighed, to a 100-cc. volumetric flask and make to volume with distilled water. Transfer a 10-cc. aliquot to a separatory funnel, add 5 cc. sodium hydroxide T.S. and extract the caffeine with successive portions of chloroform until the residue gives no test for alkaloids with iodine (T.S.). Pass the chloroform solutions through a filter which has previously been moistened with chloroform and wash the stem of the funnel and filter with a few cc. of the solvent to remove any adhering caffeine. Evaporate the combined chloroform solutions on a water-bath, and dry the residue of anhydrous caffeine to constant weight at 80° C.

Assay for Sodium Salicylate.—Transfer the aqueous liquid from which the caffeine has been removed by the above assay for caffeine to a 500-cc. glass-stoppered Erlenmeyer flask, rinsing the separatory funnel with small portions of distilled water. Also wash the filter and funnel used in the caffeine determination with small portions of water, adding the washings to the 500-cc. flask. Add sufficient distilled water to make the volume in the flask about 100 cc. Add 50 cc. 0.1*N* Bromine solution, 10 cc. of hydrochloric acid, then stopper and shake for one minute, then at intervals for thirty minutes. Add 10 cc. of 15 per cent potassium iodide solution, stopper and shake for five minutes. Titrate the liberated iodine with 0.1*N* sodium thiosulphate solution, using starch T.S. as indicator.

Each cc. of 0.1*N* bromine is equivalent to 0.002668 Gm. $NaC_7H_5O_3$.

Experimental.—According to National Formulary VI *Bulletin*, page 325, the formula and