STUDIES ON CENTAUREA BEHEN LINN. (COMPOSITAE). PART II

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The alcohol, water and piperidine extracts of *Centaurea behen* Linn. have been investigated. A glucoside, m.p. (dec.) 210-285°C., and $[\alpha]_D^{30} = -48°$ (0.5% solution in tetrahydrofuran), has been isolated from the alcohol extract. On hydrolysis the glucoside yielded glucose and the new centaurea sterol A (C_{27-28} H_{44-48} O), m.p. 133-134°C., $[\alpha]_D^{25} = -31°$. The alkaloid was present in the plant in a very small amount and did not yield sufficient material to be characterised. The reducing sugars were obtained in 23.4% yield from the aqueous extract. From the residual roots, inulin was obtained up to 38% of the fresh roots.

The alleged therapeutic importance of Centaurea behen roots in jaundice, diabetes and calculus affections cannot be attributed to the compounds isolated from the petroleum ether extract and discussed in Part I.[1] On the other hand the earlier workers, as mentioned in Part I, also failed to isolate any well-defined compound to which the therapeutic properties of Centaurea behen roots can be assigned. In this second part, the exhaustively petroleum-ether-extracted roots have been extracted with alcohol, alcohol containing 2% hydrochloric acid, cold water and finally with piperidine, and the products obtained may perhaps throw some light on the pharmacological efficaciousness of the plant.

The alcoholic extract furnished a large amount of a dark brown honey-like residue. The first two extractions on concentrating deposited a colourless amorphous globule-shaped substance which was purified by washing with petroleum ether and hot alcohol. Recrystallisation three times from tetrahydrofuran led to the pure globule shaped substance showing a constant rotation of $[\alpha]_D^{30} = -48°$ in 0.5% tetrahydrofuran solution. Found: C, 72.1; H, 10.38; O, 17.27 which fits in the range of C_{33-35} $H_{54-60}O_6$. It did not melt but decomposed slowly between 210-285°C. It was soluble in hot tetrahydrofuran, hot dimethyl formamide, pyridine, piperidine, very sparingly so in alcohol and acetone, and insoluble in petroleum ether, benzene, chloroform, ethyl acetate, carbon tetrachloride and water. With concentrated sulphuric acid it gave red colouration which intensified on warming but on further heating on a water bath the substance charred. After hydrolysis with 2N hydrochloric acid it gave needle shaped crystals and the solution reduced Fehling's solution which indicates a glucoside.

Hydrolysis of the glucoside with hydrochloric acid gas in alcohol led to a colourless sterol, m.p. 133-4°C., which crystallized in the form of leaflets from alcohol or acetone and in the form of needles from petroleum ether or ethyl acetate. The specific rotation was constant at $[\alpha]_D^{25} = -31°$ in 1% chloroform solution. The analysis C, 83.61; H, 11.8; O, 4.41 would agree best with the formula C_{27-28} $H_{44-48}O$. The difference between the formula of the glucoside and that of its aglucone indicates the sugar part to be a hexose which was proved to be glucose by paper chromatographic analysis as described in the experimental part. The infrared spectrogram of the sterol is given in Fig. 1. The positive Lieberman Burchard test, digitonin test and decolourisation of potassium permanganate in alkaline solution and bromine water indicated a sterol having a hydroxyl group in 3β-position and a double bond. Comparison of centaurea sterol and its acetate with other similarly melting sterols and their acetates, known up to 1957 is given in Table 1.

The alcoholic extract gave positive alkaloidal tests, but not in encouraging amounts. Efforts were made to isolate the little amount of the alkaloid present by solvent extraction and by precipitation with Dragendorff's reagent. The decomposition of the latter with hydrogen sulphide or with silver oxide was not successful.

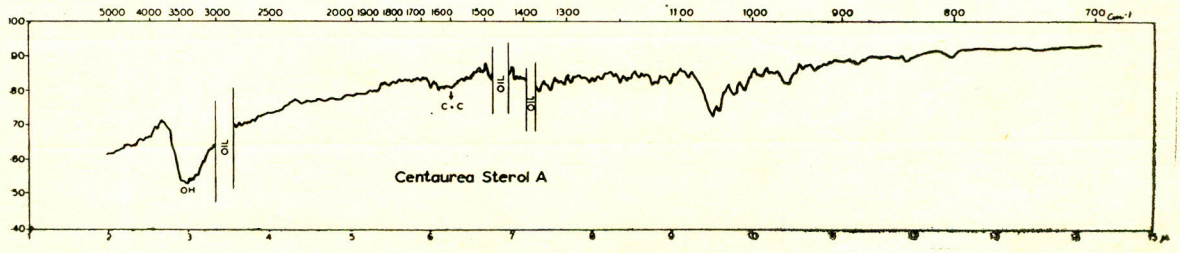

Fig. 1.—Infrared spectrogram of centaurea sterol A.

Subsequently the roots were again extracted with alcohol but 2% hydrochloric acid was added to get actually a higher percentage of alkaloid. Again large quantities of a substance came out in the blackish extract which on heating during concentration deposited huge amounts of charcoal which actually filled the distillation flasks completely. Only sugars could behave like that. Therefore, the alcohol-extracted roots in new experiments were exhaustively extracted with cold water yielding 21% of brownish residue by weight of the petroleum ether and alcohol-extracted roots. It contained 24% reducing sugars in organic salts but no alkaloid. This amount of sugar, however, could not account for the large amount of sugar coal received with alcohol containing 2% hydrochloric acid. However, the glucoside being insoluble in alcohol and water could still be expected to be present in the roots. Therefore, they were subsequently soxhleted with tetrahydrofuran, a solvent for the glucoside. But only a little amount of oily substance was obtained, from which no glucoside could be isolated. By a test extraction with cold piperidine, however, a globule-shaped white substance could be observed in fairly large amounts specially on adding alcohol to the piperidine extract. Thus 54.3% by weight of the roots extracted with petroleum ether, alcohol and water and dried afterwards were obtained which equal to 37.8% by weight of the fersh roots. This substance was quite different from centaurea glucoside. 2N Sodium hydroxide and hot 2N hydrochloric acid dissolve it easily due to hydrolysis. Ten times dissolved in hot water and precipitated with an excess of alcohol. A preparation of $[\alpha]_D^{26} = -34°$ in 1% aqueous solution was obtained. Shrinking and softening starts at 160-195°C., decomposition between 195-210°C.,

TABLE 1.—COMPARISON OF CENTAUREA STEROL A AND ITS ACETATE WITH SIMILAR STEROLS AND THEIR RESPECTIVE ACETATES KNOWN UP TO 1957.

Sterol	Centaurea sterol A	Sterol form Beach Bark[2]	Slanu-tosterol[3]*	Yasudes-terol[4]	Raphanis-terol[5]*	Verosterol[6]*	Matsusterol[7]	Pectsterol[8]	β-Sitosterol[9]
Formula	$C_{27-28}H_{44-48}O$	$C_{24}H_{40}O$	$C_{26}H_{42}O$	$C_{27}H_{46}O$	$C_{27}H_{46}O$	$C_{27}H_{46}O$	$C_{27}H_{46}O$	$C_{27}H_{46}O$ or $C_{29}H_{50}O$	$C_{29}H_{50}O$
Crystalline shape	Leaflets (alcohol or acetone) or needles (pet. ether or ethyl acetate)	Leaflet or needles	—	Scaly (methanol)	Needles (alcohol)	Leaflets	Needles (methanol)	—	Needles or leaflets
M. p.°C	133-34	134	136-7	134-5	136	135-6	130-1	134-7	137—7.5
Rotation	$[\alpha]_D^{25} = -31°$ in 1% chloroform solution	$[\alpha]_D^{18} = -31.3°$ (cholroform)	—	—	$[\alpha]_D^{20} = -32.2°$ (chloroform)	$[\alpha]_D^{15} = -33.6°$ (chloroform)	$[\alpha]_D^{15} = -33.5°$ (chloroform)	$[\alpha]_D^{28} = -34.7°$ (chloroform)	$[\alpha]_D^{24} = -36.7°$ (chloroform)
Liebermann Burchard test	Positive. Violet-Blue-Green	Positive. Violet-Dark Green	Positive	Positive (Blue colour)	Positive	—	Positive		Positive
Digitonin test	Positive	Positive	—	Positive	—	—	—	—	Positive
Double bond reactions	Positive	—	Positive	—	Positive	—	—	—	Positive
Crystalline shape of the acetate	Leaflets or needles	—	—	—	Needles (alcohol)	Needles (Acetic anhydride)	—	—	Elongated rectangular plates
M. p. of acetate°C	122-3	121-2	128	123-123.5	125	122-4	117	136.5-38	Partly at 122-3 and completely at 127
$[\alpha]_D$ of the acetate	$[\alpha]_D^{27} = -36°$ (1 % in chloroform)	$[\alpha]_D^{18} = -32.4$ (chloroform)	—	—	—	—	$[\alpha]_D^{15} = -20.9°$	—	—

*These three sterols are probably impure β-sito sterols, according to A. George.[10]

and complete charring at 210-212°C. It was proved to be a polysaccharide by charring with concentrated sulphuric acid, spot tests according to Feigl and positive Fehling's solution test after boiling with acid. The assumption to be inulin could be confirmed by paper chromatographic analysis of the hydrolysate of the sample in comparison with the hydrolysate of B. D. H. inulin and pure glucose. Furthermore the acetyl derivatives of the sample and B.D.H. inulin were prepared by Haworth and Streight's method.[11] In order to get the sample and its acetyl derivative of constant optical rotation, it was necessary to repeat the purification several times as it is the case with other poly-fructosans and their derivatives.[12] Comparison of centaurea inulin with B. D. H. inulin and the inulin with constant optical rotation according to the literature and their respective acetyl derivatives is given in Table 2.

Inulin is of commercial importance. It is used as a starting material for levulose and an ingredient of diabetic breads and food preparations due to the fact that it consists mainly of fructose being easily digestible by the diabetic patient. Inulin nitrate is used as an ingredient of detonator composition, as a sensitizer in dynamites and as an ingredient of lacquers.[13] Centaurea behen roots may therefore, become a useful source of inulin. Not knowing when the sample used in the present investigation was harvested and because the inulin content of plants varies considerably having its maximum in autumn and a minimum in spring time, the resulting percentage of 37.8% on the undried fresh roots might not represent the highest available amount. There are several other plants which contain high percentages of inulin, e.g., pyrethrum contains 57.7% at its highest; Inula 44% in autumn and 19% in spring; Arnica roots 9-7%; Helianthus tubers, Macrophyllus and Arctium lappa 20-50%; Taraxacum in October 24% and in March 1.74%; and Dahlia tubers 9.7 - 13.4%.[14] Of these, pyrethrum seems to

TABLE 2.—COMPARISON OF CENTAUREA INULIN AND ITS ACETYL DERIVATIVE WITH B. D. H. INULIN, PURE INULIN ACCORDING TO THE LITERATURE AND THEIR ACETYL DERIVATIVE.

Properties	Physical appearance	Method of isolation	Purification	Solubilities	M. p.	Sp. Rotation	Products of hydrolysis
Centaurea inulin (purified 10 times)	White amorphous powder	Extraction with piperidine or hot water and precipitation with excess of alcohol.	Dissolving in hot water and precipitation with alcohol	Similar	Shrinking, softening and foaming at 160-195°C. slow decomposition 195-210°C. complete dec. with charring 210-212°C.	$[\alpha]^{26}_{D} = -34°$ (1% aqueous solution)	Fructose and a very small amount of glucose.
B.D.H. inulin	,,	,,	,,	,,	Shrinking, softening and foaming at 155-195°C. slow decomposition 195-205°C. Complete dec. and charring 205-7°C.	$[\alpha]^{30}_{D} = -30°$ (1% aq. solution)	,,
Inulin according to the literature	,,	,,	,,	,,	—	$[\alpha]^{20}_{D} = -39°$ (in water)	Fructose and glucose. (12:1)
Acetyl derivative of c. inulin (purified 10 times)	White amorphous	Haworth or streight's method	Dissolving in chloroform or benzene & precipitating with pet. ether	,,	Shrinking, swelling and foaming at 65-115°C. Viscous at 115°C. melts at 140-50°C. to a colourless clear melt	$[\alpha]^{24}_{D} = -41°$ (1% chloro-solution)	—
Acetyl derivative of B. D.H. inulin (purified twice only)	,,	,,	,,	,,	Shrinking, swelling and foaming at 60-100°C., starts becoming viscous at 100°C. and melts at 120-30°C.	$[\alpha]^{24}_{D} = -30°$ (1% chloroform solution)	—
Inulin acetate according to literature	,,	,,	,,	,,	—	$[\alpha]^{20}_{D} = -34°$ (1.5% chloroform solution)	—

have the highest percentage of inulin but it cannot be made available because to win pyrethrum for insecticidal purposes only the flowers of this perennial plant are used continuously.[15] Actually inulin is prepared from Dahlia tubers by extraction with hot water.

Experimental

1. Extraction with Alcohol.—Centaurea behen roots, which had been exhaustively extracted with petroleum ether, were extracted with alcohol twelve times in the first experiment and seventeen times in the second experiment.

Experiment 1: 1.6 kg. of the roots, on extraction with 15 litres of distilled alcohol, yielded 105 g. of solvent-free extract (6.56% of the fresh roots).

Experiment 2: 7 kg. of the roots, on extraction with 76.5 litres of distilled alcohol, yielded 550 g. of solvent free extract (7.86% of the fresh roots).

2. Isolation of the Centaurea Glucoside.—11.5 litres of the first and second extracts of the second experiment, concentrated to about 700 ml., were kept overnight. A colourless amorphous substance settled down besides a little quantity of an oil. The amorphous substance was filtered under suction, washed with alcohol and dried on a porous plate. Yield of the crude product was 3 g. Some of the oily impurities were removed by shaking twice with petroleum ether and filtering. More of the impurities were subsequently removed by repeatedly heating with alcohol, cooling and filtering. The yield dropped down to 2 g. (0.03% of the fresh roots). This pretreated material was finally purified by recrystallising thrice from tetrahydrofuran wherefrom it crystallises in the form of globules. They were soluble in piperidine, pyridine, hot dimethyl formamide, hot dioxane, tetrahydrofuran, very sparingly so in alcohol and acetone and insoluble in petroleum ether, chloroform, carbon tetrachloride, ethyl acetate and water. The glucoside did not have any m.p. but decomposed slowly. At 210°C. it became light brown, at 235°C. brown, at 246°C. dark brown and finally it charred between 260-285°C. On treatment with concentrated sulphuric acid it became red. The colour intensified on warming and then the substance charred on prolonged heating on a water bath. With Liebermann-Burchard solution it gave blue colour which changed to green after a few minutes. It did not reduce Fehling's solution but, after heating with a few drops of 2N hydrochloric acid, there was at once reduction. The rotation ni 0.5% tetrahydrofuran solution was constant at $[\alpha]_D^{30} = -48°$. Found: C, 72.71; H, 10.38; O, 17.27 which fits into the range of $C_{33-35}H_{54-60}O_6$.

3. Hydrolysis of the Glucoside to Centaurea Sterol A and Glucose.—400 mg. of the pure glucoside was refluxed with 40 ml. of alcohol on a water bath and dry hydrochloric acid gas passed through for about 20 minutes until the whole of the glucoside went in solution. Then the boiling was continued for about 15 minutes without passing hydrochloric acid gas. On cooling, the colourless needles of the aglucone separated. The whole was brought to dryness at about 50°C. under vacuum and the last traces of hydrochloric acid removed over potassium hydroxide in a vacuum desiccator. On adding water and petroleum ether to the residue, the aglucone went into the ether layer while the glucose was dissolved in water. The two layers were separated. The petroleum ether layer was washed with water, the solvent removed and the resulting aglucone crystals dried under vacuum. Yield, 268 mg.; m.p., 125-28°C. The crude aglucone was passed through a column 1 cm. × 15 cm. containing 20 g. of alumina (Brockman). Petroleum ether and ethyl acetate were used as eluants successively. With petroleum ether, only the small amount of impurities were removed. The aglucone which came out with ethyl acetate was collected in three fractions of 4 ml. each. First fraction melted at 127-29°C., second at 128-30°C., and the third one at 130-32°C. The first two fractions were recrystallised from alcohol. Thus obtaining the purity of the third fraction m.p. 130-32°C. mixed with fraction 3 and recrystallised twice more from alcohol, the m.p. was constant at 133-34°C. It was soluble in petroleum ether, ethyl acetate, acetone, tetrahydrofuran, chloroform, carbon tetrachloride, benzene, dioxane, sparingly so in alcohol, dimethyl formamide and insoluble in water. The aglucone crystallised in the form of needles from petroleum ether or ethyl acetate and in the form of leaflets from alcohol or acetone. With Liebermann Burchard solution the product gave a violet colour which changed through blue into green within a few seconds. Potassium permanganate in alkaline solution and bromine solutions were decolourised. Positive digitonin test indicated the presence of a hydroxyl group in 3β-positon. The specific rotation of 1% solution in chloroform was constant at $[\alpha]_D^{25} = -31°$. Found: C, 83.61; H, 11.8; O, 4.41 which agrees best with the formula $C_{27-28}H_{44-48}O$. The infrared spectrogram is shown in Fig. 1. For comparison with similar sterols, see Table 1.

4. Acetylation of the Centaurea Sterol A.—100 mg. of the sterol was refluxed with 3 ml. of acetic anhydride and two drops of pyridine for about

six hours. The solvent was removed at 50°C. under vacuum and the product dried in a vacuum desiccator over phosphorous pentoxide. Yield, 112 mg.; m.p., 117-20°C. The crystalline aglucone acetate was easily soluble in petroleum ether, ethyl acetate, chloroform, benzene, cyclohexane, butanol, dioxane, carbon tetrachloride, sparingly so in alcohol, acetone, dimethyl formamide and insoluble in water. The crystals were leaflets from acetone or alcohol and needles from ethyl acetate or methyl alcohol. After four recrystallisation with ethyl alcohol, the m.p. was constant at 122-23°C. The rotation of a 1% solution in chloroform was also constant at $[\alpha]_D^{27} = -36°$.

The water layer, after the hydrolysis of glucoside, reduced Fehling's solution. It was concentrated at 50-60°C. under vacuum to syrupy consistency. Yield, 140 m.g.

5. Identification of Glucose by Paper Chromatographic Analysis.—Whatman filter paper No. 1 of 18-inch length and 6-inch width was divided into two equal parts length-wise and a line perpendicular to these parts was drawn leaving four-inch space on one side of the paper. In the middle of one of these parts and on the perpendicular line 5 drops of an approximately 0.5% solution of the sample were placed with the help of a fine capillary tube in such a way that the next drop was placed when the foregoing one had dried up. Similarly 5 drops of approximately 0.5% solution of pure glucose were placed in the middle of the second part of the paper on the perpendicular line. The drops were not allowed to spread into a circle of more than 5 mm. diameter. Touching the paper with hand was also avoided. The paper was then hanged into the chromatographic chamber with its upper part in the boat. The perpendicular line was in the horizontal position.

The solvent was prepared from butanol, acetic acid and water which were taken in the ratio of 4:1:5, respectively, in a separating funnel and shaken thoroughly several times for about 15 minutes. After separation into two different layers, the lower water layer was poured into the trough of the chamber and the upper organic solvent layer was dropped into the boat with the help of a dropping funnel. The chamber was kept air tight. After 48 hours the paper was taken out, dried in air and sprayed first with approximately 0.5% aqueous silver nitrate solution in acetone and then with 2-3% aqueous sodium hydroxide solution in alcohol. After drying the spots were made permanent by washing the paper with dilute ammonia. The experiment was repeated once, but in this case the paper was kept in the chamber for 96 hours. In both these experiments the distance travelled by the sample spot was exactly equal to that of the pure glucose. This shows that the sample had the same RF value as glucose.

6. Attempt to Isolate the Alkaloid. Precipitation with Dragendorff's Reagent.—300 g. of the sovlent-free extract was dissolved in a minimum volume of 2N acetic acid and filtered from a small amount of insoluble material. To the filtrate Dragendorff's reagent was added until the precipitation was complete. The orange precipitate was filtered, washed with distilled water and dried in a vacuum desiccator over phosphorous pentoxide. The complex precipitate was 9 g. (3% of the extract and 0.21% of the fresh roots).

Method 1: 2 g. of the complex was treated with acetone containing a few ml. of 2N acetic acid. Some of the precipitate was insoluble. Hydrogen sulphide was passed through this suspension as long as there was some precipitation, and then filtered. The excess of hydrogen sulphide was removed with the solvent at 60°C., and the acidic residue alkalified with ammonium hydroxide was brought to dryness at 60°C. under vacuum. The residue was 1.956 g. Excess of 2N sodium hydroxide was added to the residue and extracted exhaustively first with ether and then with chloroform. Both ether and chloroform extracts were dried over anhydrous sodium sulphate, filtered and the solvents removed. The ether extract yielded 46 mg. of an oily material which gave no alkaloidal tests. The chloroform extract yielded 18 mg. only and gave positive alkaloidal tests. It was reddish brown and viscous mixed with some crystalline substance in it. It was sparingly soluble in petroleum ether, acetone, ethyl acetate, alcohol, chloroform, benzene and insoluble in water. From acetone needle-shaped crystals were obtained on the slide but the amount was too small for further processing. The extract was bitter in taste.

Method 2: To 1 g. of the complex suspended in 80 ml. of moist acetone, 1.5 g. of freshly prepared and dried silver oxide together with about 20 glass beads were added. The flask was shaken for about five hours. It was filtered and washed with acetone until nothing came out any more. After removing the solvent 94 mg. of an oily extract was obtained which gave positive alkaloidal tests but no crystals with any solvent or acid could be observed. The residue was then extracted exhaustively with chloroform. The solvent removed when only 8 mg. of a needle shaped crystalline substance remained which gave positive alkaloidal tests. Finally the residue was extracted with alchohol which yielded 49 mg. of a material

free from alkaloid.

This experiment was repeated with 4 g. silver oxide. After exhaustive extraction with acetone 134 mg. of residue was obtained. It gave positive alkaloidal test but no crystals with any solvent or acid. Subsequent extraction with chloroform did not yield any crystalline substance.

7. *Extraction with Water.*—Centaurea behen roots, which had been extracted exhaustively with petroleum ether and alcohol, were dried in air in the shadow for about 2 hours. 100 g. of these roots had to be extracted six times with 250 ml. of distilled water each time at room temperature until no substance went in solution any more. On bringing to dryness in vacuum at 60-70°C., 21 g. of a brown extract having a smell of burnt sugar was obtained corresponding to 21% of the petroleum ether and alcohol extracted roots.

The sweet tasting extract was easily soluble in water, sparingly so in dimethyl formamide, acetic anhydride and insoluble in methanol, ethyl acetate, petroleum ether, chloroform, tetrahydrofuran and piperidine. It did not contain any alkaloid but 23.4% reducing sugars, determined quantitatively by Fehling's solution method. It also contained inorganic salts.

8. *Extraction of Inulin with Piperidine.*—The roots, which had been exhaustively extracted with petroleum ether, alcohol and water, were dried completely. 60 g. of the dried roots were kept standing with 150 ml. of piperidine for two days at room temperature. The clear yellowish extract was filtered under suction and the extraction repeated 6 times. The combined extracts were precipitated with alcohol, the inulin separated as a white powder. It was filtered under suction, washed with alcohol in order to free it from piperidine and dried under vacuum. Yield, 32.572 g. = 54.3% of the roots extracted with petroleum ether, alcohol and water, which equals to 37.8% of the fresh roots with about 11% moisture. The inulin can be further purified by dissolving in hot water and precipitating with excess of alcohol. This process was repeated ten times when a preparation having $[\alpha]_D^{26} = -34°$ in 1% aqueous solution was obtained. Shrinking and softening starts at 160-195°C. Decomposition with colour change starts at 195-210°C. and complete charring at 210-212°C. It was easily soluble in hot water, hot pyridine, warm piperidine, hot dimethyl formamide, sparingly so in cold pyridine, cold dimethyl formamide and cold piperidine, nearly insoluble in cold water and insoluble in petroleum ether, tetrahydrofuran, benzene, chloroform, ethyl acetate, carbon tetrachloride, cyclohexane, dioxane, butanol and alcohol. 2N sodium hydroxide and hot 2N hydrochloric acid dissolve also easily due to hydrolysis. The confirmation of being a polysaccharide was done by positive Fehling's solution test after hydrolysis and spot tests according to Fig. 1. The Fehling's solution test was also slightly positive with a little reduction on boiling even before hydrolysis.

9. *Hydrolysis of the Centaurea Inulin and Paper Chromatographic Analysis of the Hydrolysates.*—To 1.g each of B.D.H. inulin and the sample from centaurea behen, 15 ml. of N sulphuric acid were added and allowed to stand for about six hours at room temperature (31°C.). During this time nearly all of both the samples had gone in solution. The sulphuric acid was precipitated completely as barium sulphate by drop-wise addition of barium hydroxide solution. After heating up to boiling it was cooled and filtered. Both the filtrates were diluted to about 0.5% concentration and compared with a 0.5% glucose solution by paper chromatography in the described manner. After 48 hours the paper was taken out, dried and sprayed first with 0.5% aqueous silver nitrate solution in acetone and then with 2-3% aqueous sodium hydroxide solution in alcohol. The spots were made permanent by washing the paper with dilute ammonia. The spots for the sample and B.D.H. inulin were exactly the same concerning wandering speed and intensity of the glucose and fructose parts, while the spot of the glucose sample was in line with the corresponding spots of the hydrolysates.

10. *Preparation of the Acetyl Derivative of C. Inulin by Haworth and Streight's Method.*—2 g. of the centaurea inulin was shaken with 20 ml. of freshly distilled pyridine in a round bottomed flask at 80-90°C. for about 45 minutes until the whole went in solution. After cooling, 4 ml. of freshly distilled acetic anhydide was added drop-wise. The flask was covered with a calcium chloride tube and heated at 80-90°C. for about 6 hours on water bath. The clear solution was then poured into 200 ml. of water. The acetate was separated out as a white and somewhat gelatinous substance sticking to the walls. The aqueous solution was decanted off and the residue washed repeatedly with distilled water, dried and purified by dissolving in hot methyl alcohol, filtering and separation on cooling. Finally it was purified by dissolving in chloroform and precipitating with petroleum ether. This process was repeated ten times and then dried over phosphorous pentoxide in a vacuum desiccator. The rotation was $[\alpha]_D^{24} = -41°$ in 1% chloroform solution. Shrinking, swelling and foaming starts between 65-115°C.

at 115°C. It becomes viscous and melts completely at 140-150°C. to a colourless clear melt. The acetyl derivative was a white amorphous powder, easily soluble in chloroform and benzene, sparingly so in methyl alcohol and insoluble in petroleum ether and water.

In the same way the acetyl derivative of B.D.H. inulin was prepared. For data see Table 2.

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