1-phosphate, followed after 10 min. by dilute iodine spray, to test for formation and find location of "priming" oligosaccharides.[12] The Schardinger dextrins, especially cyclohexaamylose, markedly inhibited phosphorylase[24] and depending on the relative amount present gave either a white zone of inhibition (low concentration) or a wide zone of inhibition with central blue-violet area of the cyclohexaamylose–iodine–potassium iodide complex (high concentration). High primer concentrations were found to reduce the extent of inhibition (see Fig. 1). With co-substrates containing keto groups (sucrose, turanose, planteose, melezitose, D-glucoheptulose) coupled products were revealed by spraying with urea or phloroglucinol in hydrochloric acid, followed by heating in the oven at 100° for about 5 min. Aucubin coupled products gave blue-green colors with the phloroglucinol–hydrochloric acid spray. Ammoniacal silver nitrate was found to be a satisfactory spray reagent for coupled products from polygalitol.

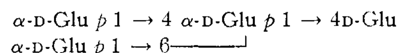
By comparing spot positions and characters with controls of the ordinary amylose series and suitable blanks it was possible to ascertain whether, and to what extent, coupling reactions had occurred.

Coupling with Radioactive Glucose.—A sample of radioactive glucose (2 mg., 0.5 mc.) was treated with cyclohexaamylose (10 mg.) and *macerans* amylase (1.25 units[4]) for 500 min. at room temperature. The whole of the digest was transferred to a sheet of filter paper and chromatographed. A radioautograph of the resulting chromatogram (Fig. 2) showed that radioactive oligosaccharides containing at least 10 D-glucose units were produced and clearly resolved. By sectioning the chromatogram individual radio-oligosaccharides were obtained for structure analysis and other work. No evidence was seen for any radioactivity in the Schardinger dextrin bands. Cyclohexaamylose appeared particularly well separated midway between G_4 and G_5. It was eluted and definitely identified by means of the iodine test.[4] In non-radioactive experiments, cycloheptaamylose was shown to have nearly the same R_f as G_5 while cyclooctaamylose fell between G_6 and G_7.

Structure of Coupled Products Derived from Radioactive Glucose.—The resolved chromatogram (Fig. 2) was autographed, sectioned and individual saccharides up to G_7 were isolated. G_2 was converted into the 1-phenylflavazole derivative by heating with 10 mg. of carrier G_2 and the required proportions of reagents[15] in a sealed capillary tube at 100° for 3 hours. Maltose-1-phenylflavazole was isolated from the reaction mixture by paper chromatography, and shown to be radioactive. Acid hydrolysis gave radioactive glucose-1-phenylflavazole and inactive glucose (separated by paper chromatography). Isolated G_3 and higher oligosaccharides were cleaved by salivary amylase forming radioactive saccharides such as G_1, G_2 and G_3. Action of soybean β-amylase on G_3 and G_5 gave inactive G_2 and radioactive G_1.

Structure of Coupled Products Derived from Sucrose.—A *macerans* amylase digest of sucrose and cyclohexaamylose was treated with 0.1 *N* NaOH at 100° for 30 min. (to remove traces of reducing oligosaccharides) and neutralized. Paper chromatography then showed the presence of a series of fructose-containing compounds of which the tetrasaccharide was a poor primer and higher members were good primers for phosphorylase (Fig. 3). Partial hydrolysis ("inversion") by 0.1 *N* hydrochloric acid at 70° for 5 min. or by yeast invertase, produced fructose and the normal series of reducing amyloöligosaccharides; no fructose-containing oligosaccharides were present.

Coupled Products Derived from Panose.—Panose[10] gave a series of coupled products which differed from other coupling series in that there were two different distinctly resolved tetrasaccharide components, and the pentasaccharide and higher components appeared to be mixtures of isomers. Partial acid hydrolysis of the tetrasaccharides and their flavazoles indicated the faster moving component to be



α-D-Glu *p* 1 → 4 α-D-Glu *p* 1 → 4D-Glu
α-D-Glu *p* 1 → 6———┘

and the slower moving component to be α-D-Glu *p* 1 → 4 α-D-Glu *p* 1 → 6 α-D-Glu *p* 1 → 4 D-Glu.

The lowest component capable of priming phosphorylase was the pentasaccharide. However, when the mixture was treated with either salivary amylase or β-amylase, the ability of the pentasaccharide to prime phosphorylase was largely destroyed, though a significant amount of reducing pentasaccharide remained. Action of an amyloglucosidase[25] on the panose coupling mixture converted it entirely into glucose and panose.

(24) D. E. Green and P. K. Stumpf, *J. Biol. Chem.*, **142**, 355 (1942).

(25) "Dextrinase," a microbial enzyme sold by the Delta Chemical Works, 23 W. 60th Street, New York 23, N. Y.

AMES, IOWA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, PRESIDENCY COLLEGE, CALCUTTA]

Bitter Constituents of the Seeds of *Corchorus olitorius* L., "Corchorgenin"—A New Cardiac-active Aglycone

BY JIBAN K. CHAKRABARTI AND NIRMAL K. SEN

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A new cardiac-active aglycone, $C_{23}H_{32}O_6$, m.p. 227° (uncor.), $[\alpha]^{21}D$ +90° (ethanol), has been isolated by chromatography of the bitter principles from seeds of *Corchorus olitorius* L. grown in West Bengal (India) and studied. Its pharmacological activity in cats, indicates that it is more potent than either of the isomeric genins, corchortoxin of *Corchorus capsularis* or strophanthidin of *Strophanthus kombe.*

During the last few years there has been a renewed interest in the chemistry of the bitter principles of jute (*Corchorus*[1]) seeds and different bitter principles have been reported. The occurrence of a bitter principle as a brown amorphous powder under the name of "corchorin" in the seeds of *C. capsularis* L. was first reported by Tsuno,[2] but our present knowledge regarding the isolation, chemistry and pharmacological properties of corchorin is mainly due to Sen.[3] He described corchorin as a glycoside, $C_{22}H_{36}O_8$, m.p. 174–175°, $[\alpha]D$ +33.4° (ethanol), which yielded glucose and corchogenin, $C_{16}H_{26}O_3$, m.p. 112–114°, on acid hydrolysis. Corchogenin according to Ruzicka[4] may be a sesquiterpene or sesquiterpenoid. Sen also isolated another bitter principle "corchoritin,"[5] $C_{12}H_{18}O_3$, m.p. 218–

(1) The two important species of *Corchorus* L. (*Tiliaceae*), *viz.*, *C. capsularis* L. and *C. olitorius* L. are grown in West and East Bengal which produce about four-fifths of the world's total jute output; the latter species is widely used as a pot-herb. The seeds of the two species can be differentiated morphologically; those of *C. capsularis* are relatively larger in size, irregular in shape and coppery red when mature, whereas the seeds of *C. olitorius* are somewhat triangular and greyish-green or bluish-black in color. In the present investigation, the seeds of *C. olitorius* L. collected from the Hooghly District (West Bengal) have been employed.

(2) Tsuno, *Monatsh. Tierheilk*, **6**, 455 (1896).

(3) N. K. Sen, *J. Indian Chem. Soc.*, **7**, 83 (1930); *Indian J. Physiol. Allied Sci.*, **2**, 1 (1948).

(4) L. Ruzicka, *Ann. Rev. Biochem.*, **1**, 594 (1932).

(5) N. K. Sen, *J. Indian Chem. Soc.*, **8**, 651 (1931).

220°, $[\alpha]_D$ −35.1° (ethanol), from the seeds of *C. capsularis* L. and described it as an hydroxy lactone. Recently, another bitter cardiac-active agent "corchortoxin"[6] was isolated from seeds of *C. capsularis* L. Corchortoxin, $C_{23}H_{32}O_6$, m.p. 247°, $[\alpha]^{18}_D$ +67.9° (ethanol), was found to be an aglycone containing four hydroxyl groups. Its cardiac action is similar, though less intense, to that of the digitalis group of genins.

In a study[7] of Egyptian *C. olitorius* the presence of a glycosidic principle was indicated in the alcoholic extract of the seeds. Eventually the so-called corchorin isolated from the seeds of both Egyptian *C. capsularis* and *C. olitorius* was reported[8] to be identical with strophanthidin. It is interesting that none of the previous workers reported strophanthidin in jute seeds which were mostly collected from different parts of Bengal. More recently, another bitter substance "corchsularin," $C_{30}H_{57}O_9$, m.p. 157°, was isolated together with corchorin from seeds of *C. capsularis* L.[9]; this corchorin is almost the same as that previously described by Sen,[3] and not identical with strophanthidin reported by Soliman.[8] Apparently jute seeds contain a mixture of glycosides derived from different aglycones, the nature of which may possibly vary with several factors, *e.g.*, climate and soil, maturity of the seeds, time between collection and chemical extraction, enzyme effect, etc. It, therefore, appeared of interest to isolate the actual heart poison from the seeds of *C. olitorius* L.

Besides raffinose,[3,8,10] to the extent of 2.5%, the ethanolic extract of the defatted seeds yielded an extremely bitter substance from which a pure, crystalline compound, m.p. 227°, $[\alpha]^{21}_D$ +90° (ethanol), was isolated by chromatographic purification. It is soluble in ethanol, methanol and pyridine; sparingly soluble in water, chloroform and benzene, and insoluble in ether. It gives a red color with a green fluorescence in concentrated sulfuric acid, but no color with ferric chloride. It is not hydrolyzed by 2% sulfuric acid, by various concentrations of mineral acids in alcohol, or by hydrochloric acid and acetone.[11] In certain cases the unchanged material is recovered; otherwise it undergoes resinification without elimination of sugar moiety. Evidently the substance is a genin and not a glycoside. The dehydrated compound has the empirical formula $C_{23}H_{32}O_6$ which is isomeric with strophanthidin and corchortoxin. But unlike strophanthidin it contains no reactive carbonyl group, since it fails to react with hydroxylamine, phenylhydrazine, etc.; in such cases almost all (99%) of the unchanged genin is recovered. The absence of a carbonyl group is also indicated by its ultraviolet absorption spectrum. Neither does it contain an alkoxyl (Zeisel determination) or free carboxyl group. The presence of one α,β-unsaturated lactone ring is shown by its positive Legal test and reduction of Tollens reagent, by alkali titration and also by its characteristic absorption (maximum 218 mμ, ethanol). The genin has an hydroxyl group which can be acylated; the monoacetate melts at 240–242°. About 97% of the acetate is recovered from the chromatographic analysis of the acylated product; this finding confirms the homogeneity of the genin. The remaining three oxygen atoms appear to be present in hydroxyl functions, one of which might account for the formation of a characteristic iso-compound.

The bioassay of the genin reveals its digitalis-like cardiac action. A typical digitalis-like effect could be demonstrated with a concentration of 5×10^{-6}; this activity is practically identical with that of ouabain. In these tests 1 mg. of the genin is equivalent to about 2 I.U. In cats its potency[12] (mean geometric LD, 0.2658 ± 0.0234 SE) indicates that it is more active than either of the isomeric genins, corchortoxin[6] or strophanthidin[13] of *S. Kombe.* Further investigation is required to establish its structural relationship to corchortoxin. The genin has been provisionally named "corchorgenin."

(6) P. Karrer and P. Banerjea, *Helv. Chim. Acta*, **32**, 2385 (1949).
(7) Amin, *et al.*, *Report Pharm. Soc. Egypt*, **6**, 12 (1934).
(8) G. Soliman and W. Saleh, *J. Chem. Soc.*, 2198 (1950).
(9) M. A. Khalique and M. Ahmed, *Nature*, **170**, 4337 (1952).
(10) H. E. Annett, *Biochem. J.*, **11**, 1 (1917).
(11) C. Mannich and G. Siewert, *Ber.*, **75**, 737 (1942).

Experimental[14]

Extraction.—The defatted seeds (3 kg.) were exhaustively extracted with ethanol (98%) in a Soxhlet for about 10–12 hours; when cold, some brownish solid material was deposited in the extraction flask; this was collected. More separated from the extract on further concentration and subsequent cooling. These deposits were combined, treated with a little hot water and clarified by filtration. The filtrate was concentrated under reduced pressure to a sirup. This was dissolved in ethanol (90%) and treated with animal charcoal to give a white crystalline product (about 2.5%) which was identified (m.p. 74–75°, $[\alpha]^{31}_D$ +102.4° (*c* 4.124, water) as raffinose.

Isolation of the Bitter Principle.—The ethanolic extract, from which the raffinose had been separated, was distilled under reduced pressure on a water-bath to remove most of the solvent, and a dark brown sirupy liquor was obtained. This was digested hot with sufficient water (4 l.), and when cold filtered from some brownish resinous matter. The filtrate was warmed, treated with excess 15% lead acetate solution (hot) in the usual way, and the excess lead precipitated with hydrogen sulfide. Upon concentration of the clear yellow filtrate on a water-bath, a brownish crystalline mass (6.5 g.) separated slowly. This was collected and washed successively with water, a little alcohol (absolute) and ether; the dried material yielded an extremely bitter white crystalline product melting at 168–170° with decomposition. This product was treated with animal charcoal in ethanol (90%) and recrystallized from dilute methanol, yielding colorless crystals, m.p. 172–175° dec.; repeated crystallization from methanol or ethanol did not change the melting point. It did not reduce Fehling reagent even after treatment with mineral acids.

Separation of Corchorgenin by Chromatography.—One hundred mg. of the product was chromatographed in purified ethyl acetate on precipitated zinc carbonate (7.6 g., Merck reagent grade). The results obtained are shown in Table I.

Upon repeated crystallization from ethyl acetate (hot), combined fractions IV–VII yielded colorless crystals with a well-defined m.p. (227°) and $[\alpha]^{21}_D$ +90° (*c* 1.0125, ethanol).

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.2; H, 7.9; mol. wt., 404. Found: C, 68.03; H, 8.15; mol. wt. (camphor, Rast), 424, (titration), 418.

Attempted Hydrolysis of Corchorgenin. (1) With Methanolic Hydrochloric Acid.—One hundred mg. of corchorgenin was dissolved in 5 ml. of methanol containing 2.5% hydro-

(12) K. K. Chen, *et al.*, *J. Pharm. Exp. Therap.*, **103**, 420 (1951).
(13) K. K. Chen and R. C. Elderfield, *ibid.*, **70**, 338 (1940).
(14) Melting points are not corrected. Unless otherwise stated the analytical specimens are dried at 110° under 0.01 mm. over phosphorus pentoxide.

TABLE I

No. of fractions[a]	Collected, ml.	Weight, mg.	Melting point, °C.
I	20	9	173–175
II	20	13	174–175
III	10	15	Soft. at 174 Melted at 224
IV	15	17	224–226
V	25	28	226–227
VI	25	7	226–227
VII	25	5	226–227

[a] All fractions were eluted with ethyl acetate except for VII which was eluted with ethyl acetate and methanol.

chloric acid and kept at 38–40° for a week. The solution was evaporated off under vacuum; the residue was taken up with water, filtered and washed with water. The filtrate did not give sugar test. The residue was recrystallized from ethyl acetate, dried and weighed (98 mg., m.p. 226–227°).

(2) **With 2% Sulfuric Acid.**—One hundred mg. of corchorgenin dissolved in 5 ml. of ethanol (absolute) was refluxed on a water-bath with 2% sulfuric acid for about 4 hours. Some resinous matter separated. The filtrate did not reduce Fehling solution nor yield an osazone.

(3) **With Hydrochloric Acid and Acetone.**—One hundred mg. of corchorgenin was treated with 10 ml. of acetone containing 3–4 drops of 0.01 *N* HCl and kept at 20–21° for 6 days. The solution was distilled off under vacuum and the residue was extracted with water and filtered. The filtrate did not give a positive test for sugar.

TABLE II

Fraction	Collected, ml.	Weight, mg.	Melting point, °C.
I	10	5	240
II	15	8	240–242
III	20	26	240–242
IV	25	38	240–242
V	25	13	240–242
VI	30	7	240–242

Corchorgenin Acetate.—Two hundred mg. of corchorgenin in 2–3 ml. of pure dry pyridine was acylated with acetic anhydride (4 ml.) at room temperature. The reaction product on crystallization from ethanol yielded corchorgenin acetate, m.p. 240–242°. This acylated product (100 mg.) was chromatographed in ethyl acetate solution through a column of zinc carbonate. All the fractions were eluted with ethyl acetate, collected and analyzed (Table II).

All these fractions (97 mg.) were combined and recrystallized from ethanol.

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.23; H, 7.66. Found: C, 67.08; H, 7.79.

This acetate was hydrolyzed with 2% methanolic $KHCO_3$ solution; the regenerated product on crystallization from ethyl acetate melted at 227°; the melting point was not depressed by admixture with corchorgenin.

Lactone Titration.—0.0946 g. of corchorgenin in 25 ml. of neutral ethanol was refluxed with 10 ml. of 0.1 *N* KOH on a water-bath for 30 min. The excess alkali was titrated with (1.1316) 0.1 *N* HCl with phenolphthalein as the indicator. The alkali used up in the experiment was 0.0127 g. of KOH; the theoretical amount for one lactone ring is 0.0131 g. of KOH.

Isocorchorgenin.—One hundred mg. of corchorgenin was treated with 10 ml. of 5% methanolic KOH, kept at room temperature for 3 hours, and then poured into water; the precipitate (15 mg.) was filtered off. The filtrate was acidified with dilute HCl to congo red and warmed to 40° for about 5 min. The resulting precipitate was filtered, washed free of acid, crystallized from dilute ethanol and dried over P_2O_5; m.p. 200°. It did not give a Legal test; it was soluble in alkali and could be reprecipitated with acid.

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.2; H, 7.9. Found: C, 67.98; H, 8.10.

Corchorgenin yielded no oxime or phenylhydrazone.

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WEST BENGAL, INDIA

[CONTRIBUTION FROM THE BOTANY DEPARTMENT, CORNELL UNIVERSITY]

The Detection of the Keto Acids of Plants. A Procedure Based on their Conversion to Amino Acids

BY G. H. N. TOWERS, J. F. THOMPSON AND F. C. STEWARD

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A method for the identification and quantitative determination of keto acids in plants has been described. The 2,4-dinitrophenylhydrazones of the keto acids are converted to amino acids by catalytic hydrogenolysis and determined by means of quantitative paper chromatography. This has been done for α-ketoglutaric, oxaloacetic, pyruvic and glyoxylic acids all of which occurred in the plant materials examined. It has been found, by the use of this method, that a number of hitherto unreported keto acids occur in plants and many of these have as yet to be identified. A key to the recognition of the unidentified keto acids that occur in certain plant materials is given and the amounts of the identifiable keto acids that occur in three unrelated types of plant material have been determined. Attention is drawn to the implications of these results.

Introduction

The α- and β-keto acids occupy a central position in modern views of carbohydrate and nitrogen metabolism in plants. Since the development of paper chromatography, much has been learned about the range of nitrogen compounds that occur in plants. It is now possible to apply similar techniques to the study of the keto acids. This is important because the keto acids may be expected to be indicators of metabolic pathways.

The keto acids concerned in the oxidative cycle in plants have been inferred largely from enzyme studies and it is doubtful whether a complete analysis has been made for the keto acids of any one plant.

The scant information regarding the keto acids of plants is the natural consequence of the low concentrations of these compounds in plants and the difficulties encountered in the application of current methods[1-4] for their identification and determina-

(1) S. M. Altman, E. M. Crook and S. P. Datta, *Biochem. J.*, **49**, lxiii (1951).

(2) D. Cavallini, N. Frontali and G. Toschi, *Nature*, **163**, 568 (1949).

(3) D. Seligson and B. Shapiro, *Anal. Chem.*, **24**, 754 (1952).

(4) A. I. Virtanen, J. K. Miettinen and H. Kuntu, *Acta Chem. Scand.*, **7**, 38 (1953).