107. *The Glucosides of* Strophanthus Emini.

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Some years ago the Director of the Imperial Institute suggested that the seeds of *Strophanthus Emini* might be considered by the Pharmacopœia Commission as an alternative to the seeds of *S. kombé*, which are alone official. With this end in view the Commission arranged for investigations of the chemistry, pharmacognosy, pharmacology, and clinical use of the drug, which have been partly reported elsewhere (Anon., *Quart. J. Pharm.*, 1935, 61; Lamb and Smith, *ibid.*, p. 71).

When the investigation was started, comparatively little was known of the glucosides of this species and the presence of strophanthin in *S. Emini* had been both denied and affirmed. While the work was in progress an important paper by Jacobs and Bigelow (*J. Biol. Chem.*, 1932—3, **99**, 521) appeared. The strophanthin which they isolated consisted of a complex mixture, which was divided into two classes according to the ease of hydrolysis by acids. The more easily hydrolysable glucosides are glucosides of an *a*-deoxy-sugar and on hydrolysis give a mixture of strophanthidin and periplogenin. The less easily hydrolysable glucosides give a monoside, $C_{30}H_{46}O_9$, soluble in chloroform, and a bioside, $C_{36}H_{56}O_{14}$, sparingly soluble in chloroform, both of which are derivatives of the same genin $C_{23}H_{34}O_5$. The last was isolated only as the trianhydrogenin $C_{23}H_{28}O_2$, isomeric but not identical with trianhydroperiplogenin. The sugar in the monoside appeared to be isomeric or identical with digitalose and the bioside contains an additional hexose, possibly glucose.

For the present investigation the seeds of *S. Emini* were supplied by the Director of Agriculture, Tanganyika, through the courtesy of the Director of the Imperial Institute. One lot of seeds, at least five years old, and two subsequent lots, specially collected and worked up soon after receipt, all gave similar results. The "strophanthin" prepared as previously described (Lamb and Smith, *loc. cit.*) consisted of a complex mixture of amorphous water-soluble glucosides which contained 3—5% of the acetyl group. In this respect it resembles the strophanthins of *S. kombé* and *S. sarmentosus*, which we examined for comparison and also found to contain acetylglucosides, the presence of which has not hitherto been recorded in strophanthin. A small portion of the glucosides was soluble in chloroform and consisted of a mixture of monosides, from which was isolated a small amount of cymarin. As the amorphous material did not lend itself to fractionation, it was subjected to enzyme hydrolysis by the method used by Jacobs and Hoffmann (*J. Biol. Chem.*, 1926, **69**, 153) in the investigation of other species of *Strophanthus*. By this means the greater part of the complex glucosides was converted into monosides soluble in chloroform. A part which was incompletely hydrolysed gave a crystalline water-soluble glucoside, which has not yet been obtained pure. It appears to consist of a mixture of a bioside formed from emicymarin (see below) and glucose and its acetate. The monosides consisted of a mixture which on crystallisation gave some initial crops which could not be separated into pure glucosides, but which on hydrolysis gave a mixture of strophanthidin and periplogenin with the sugar cymarose. Subsequent crops gave a new glucoside which, since it seems to be characteristic of *S. Emini*, is named *emicymarin*.

Emicymarin, $C_{30}H_{46}O_9$, is a typical monoside of the heart poison group. Like the other cardio-tonic glucosides, it is a lactone and gives the Legal reaction for Δ^{β}-unsaturated lactones (Jacobs, Gustus, and Hoffmann, *J. Biol. Chem.*, 1926, **67**, 333; **70**, 1). It does not form an oxime. When the double bond is saturated either by reduction or by isomerisation, the respective products no longer give the Legal reaction. It forms a *diacetate*. Emicymarin is unchanged by the gentle conditions of hydrolysis which hydrolyse glucosides, such as cymarin and periplocymarin, which contain α-deoxy-sugars. More drastic conditions of hydrolysis give rise to a trianhydrogenin, $C_{23}H_{28}O_2$, identical with trianhydroperiplogenin, a *monoanhydrogenin*, $C_{23}H_{32}O_4$, and a sugar, digitalose, which has been obtained crystalline for the first time and identified by conversion into digitalonolactone.

Jacobs and Bigelow (*loc. cit.*) have already suggested that the glucosides which they isolated might be glucosides of *allo*periplogenin. The seeds of *S. Emini* have therefore been subjected to the process carried out by Jacobs (*J. Biol. Chem.*, 1930, **88**, 519) with *S. kombé*

seeds. After this allomerisation process a mixture of sparingly soluble monosides was obtained, from which the monoside of Jacobs and Bigelow was isolated in addition to the above-mentioned glucoside. The same monoside was obtained when emicymarin was subjected to the action of the marc from *S. Emini* seeds and it is therefore *allo*emicymarin.

*allo*Emicymarin on hydrolysis gave the trianhydrogenin $C_{23}H_{28}O_2$ described by Jacobs and Bigelow and in addition a monoanhydrogenin, $C_{23}H_{32}O_4$. The sugar is identical with that from emicymarin, *viz.*, digitalose.

Experimental.

The crushed seeds, freed from fat by light petroleum, were extracted with 90% alcohol. The extract was treated with basic lead acetate and after filtration the excess of lead was removed with hydrogen sulphide. The liquor was evaporated nearly to dryness in a vacuum. The residue was dissolved in water and extracted with chloroform, the extract dried (anhydrous sodium sulphate) and concentrated, and the glucosides precipitated by the addition of light petroleum. The chloroform-soluble fraction amounted to 0·09% of the fat-free seeds. The aqueous liquor was saturated with ammonium sulphate. The water-soluble glucosides separated as a sticky mass, which was collected and extracted with dehydrated alcohol. After filtering from ammonium sulphate, the alcoholic liquor was evaporated to dryness under reduced pressure. The residual amorphous water-soluble glucosides amounted to 4·2% of the fat-free seeds and contained approximately 4% of acetyl.

Isolation of Cymarin.—The chloroform-soluble glucosides from 7 kg. of fat-free seeds after two crystallisations from methyl alcohol gave 2·25 g. of cymarin identical with cymarin from *S. kombé*. It melted at 142—150° and gave a bluish-purple Keller reaction. The anhydrous glucoside had $[\alpha]_{5461}^{20°}$ + 45·0°, $[\alpha]_{D}^{20°}$ + 38·4° (c = 1·5 in absolute alcohol). Anhydrous cymarin prepared from *S. kombé* seeds had $[\alpha]_{5461}^{15°}$ + 45·5°, $[\alpha]_{D}^{17°}$ + 39·2° (c = 1·9 in absolute alcohol) (Found for the dried substance: C, 65·6; H, 8·2; OMe, 6·0. Calc. for $C_{30}H_{44}O_9$: C, 65·6; H, 8·1; OMe, 5·7%). For further comparison the cymarin from each source was converted into strophanthidin. Alone or when mixed, both specimens melted at 161—165°. The strophanthidin from *S. Emini* had $[\alpha]_{5461}^{15°}$ + 45°, $[\alpha]_{D}^{15°}$ + 38° (c = 1·14 in 95% alcohol), and that from *S. kombé* had $[\alpha]_{5461}^{20°}$ + 48°, $[\alpha]_{D}^{20°}$ + 41° (c = 1·04 in 95% alcohol). The identity was confirmed by conversion into the methylal of the dianhydro-derivative by the method described by Jacobs and Bigelow (*loc. cit.*). Alone or mixed, both specimens melted at 253°.

Enzyme Hydrolysis of the Amorphous Water-soluble Glucosides.—The enzyme was prepared from *S. Emini* seeds by the method given by Jacobs and Hoffmann (*J. Biol. Chem.*, 1926, **69**, 157). A mixture of the amorphous glucosides (10 g.) and crude enzyme (5 g.) in water (700 c.c.) at p_H 5·6 was left at 37° for 9 days. The reducing sugar formed was equivalent to 2·38 g. of glucose determined by the Bertrand micro-method (*Mikrochemie*, 1931—2, **10**, 133). The mixture was treated with five volumes of alcohol, filtered through kieselguhr, and evaporated to small bulk under reduced pressure. The residue was extracted with chloroform. The aqueous layer was saturated with ammonium sulphate, which precipitated the water-soluble glucosides (2·4 g.). These were redissolved in water, and the solution partially saturated with ammonium sulphate. On standing, a water-soluble crystalline glucoside separated as a mass of microneedles. The chloroform-soluble glucosides (4·3 g.) were crystallised from methyl alcohol. The first crops yielded 2·1 g. of a mixture of glucosides, and the mother-liquors 0·55 g. of crude emicymarin. *The easily hydrolysed monosides.* The mixture referred to above had a lower rotation than pure cymarin and judging by the analytical results was a mixture of two similar glucosides. Various attempts to isolate the pure glucosides failed. The material was hydrolysed by the method used by Windaus and Hermanns (*Ber.*, 1915, **48**, 979). The aglucone mixture so obtained also could not be separated by crystallisation, but separation was effected by formation of the oxime. The aglucone, hydroxylamine hydrochloride, and fused sodium acetate (0·01 mol. of each) in 50 c.c. of alcohol were boiled for 6 hours. When the liquor was concentrated, crude periplogenin separated; it was crystallised from ethyl acetate, dilute alcohol, and methyl alcohol until its properties were constant. They then agreed with those described by Jacobs and Hoffmann for the periplogenin from *Periploca graeca* (*J. Biol. Chem.*, 1928, **79**, 527). The anhydrous aglucone had $[\alpha]_{5461}^{23°}$ + 37·9°, $[\alpha]_{D}^{23°}$ + 31·4° (c = 1·32 in alcohol). Jacobs and Hoffmann give $[\alpha]_{D}^{27°}$ + 31·5° (c = 1·04 in alcohol) (Found for the substance dried at 110° in a vacuum: C, 70·9; H, 8·7; OMe, nil; N, nil; M, by lactone titration, 358. Calc. for periplogenin, $C_{23}H_{34}O_5$: C, 70·7; H, 8·8%; M, 390). The benzoate separated from dilute alcohol in

glistening wedge-shaped prisms, m. p. 227° to 233° depending on the rate of heating. Jacobs and Hoffmann describe it as glistening wedges " which when heated rapidly melt at 235."

The strophanthidin oxime contained some impurity, which was removed by repeated crystallisation from alcohol. It crystallised in anhydrous rhombic prisms identical in appearance with strophanthidin oxime from *S. kombé*. It melted at 260—270°. $[\alpha]^{24°}_{5461}$ + 82·5°, $[\alpha]^{24°}_{D}$ + 69·8° (c = 1·00 in pyridine). Jacobs and Heidelberger give $[\alpha]^{23°}_{D}$ + 71·3° in pyridine (*J. Biol. Chem.*, 1922, **54**, 253) (Found: C, 65·5; H, 7·9; N, 3·4. Calc. for strophanthidin oxime, $C_{23}H_{33}O_6N$: C, 65·8; H, 7·9; N, 3·3%).

Cymarose was isolated after removal of the aglucones by the method of Windaus and Hermanns (*loc. cit.*). It separated from dry ether in needles, m. p. 82—84°. The melting point varies somewhat with the rate of heating. It was not lowered when the sugar was mixed with cymarose obtained from *S. kombé* cymarin. The substance showed a slight mutarotation. The end rotation was $[\alpha]^{24°}_{5461}$ + 61·4°, $[\alpha]^{24°}_{D}$ + 52·4° (c = 2·04 in water). For a specimen of cymarose from *S. kombé* cymarin, $[\alpha]^{24°}_{5461}$ + 60°, $[\alpha]^{24°}_{D}$ + 54° (c = 2 in water). Jacobs and Hoffmann (*J. Biol. Chem.*, 1926, **67**, 617) give m. p. 91° and $[\alpha]^{21°}_{D}$ + 53·4°. The sugar gave the blue colour in the Keller reaction typical of α-deoxy-sugars (Found: C, 51·6; H, 8·6; OMe, 18·9. Calc. for cymarose, $C_7H_{14}O_4$: C, 51·8; H, 8·7; OMe, 19·1%). The cymarose was not isolated in a sufficiently good yield to prove that it was the only sugar present, but the fact that it crystallised suggests that no other sugar was there.

Emicymarin, $C_{30}H_{46}O_9$, after repeated crystallisation from methyl alcohol separated in rosettes of small stout needles or separate prisms. After being dried, it sintered at 160° and melted indefinitely about 207°. The anhydrous substance has $[\alpha]^{20°}_{5461}$ + 15·8°, $[\alpha]^{20°}_{D}$ + 12·8° (c = 2·5 in absolute alcohol). It is readily soluble in ethyl alcohol, methyl alcohol, acetone, and chloroform, but sparingly so in water. Crystallised from methyl alcohol, it contains varying amounts of water and methyl alcohol of crystallisation, the latter being lost slowly on exposure to air. It crystallises from cold dilute alcohol with four molecules of water (Found: H_2O, 11·6. $C_{30}H_{46}O_9,4H_2O$ requires H_2O, 11·6%). It crystallises from ethyl acetate free from solvent. It gives a positive Legal reaction, and with the Keller reaction a ring which with reflected light is brown below and green above. The glucoside dissolves in concentrated sulphuric acid to give an orange solution. It has a bitter taste (Found for the glucoside dried at 120° in a vacuum: C, 65·4; H, 8·6; OMe, 5·8; M, by lactone titration, 517. $C_{30}H_{46}O_9$ requires C, 65·4; H, 8·4; OMe, 5·6%; M, 550).

The glucoside could not be hydrolysed by 30% alcohol containing 7% of hydrochloric acid. It was hydrolysed by boiling 2% hydrochloric acid to digitalose and a resinous aglucone which could not be crystallised. The latter or the glucoside itself, when treated with hot 5% hydrochloric acid, gave a mixture of crude anhydrogenins.

Trianhydroemicymarigenin, $C_{23}H_{28}O_2$.—Emicymarin methyl alcoholate (6·8 g.) in warm alcohol (60 c.c.) was added to 1200 c.c. of boiling 5% hydrochloric acid. A somewhat sticky yellow solid separated immediately. After boiling for 2 minutes, the solution was cooled and kept for some time. The separated solid was recrystallised from alcohol. The crystals (1·96 g.) consisted of a mixture of the trianhydrogenin and another compound similar in properties but having a lower rotation and containing a smaller percentage of carbon. The latter compound has not been obtained pure, but may be the dianhydro-derivative. On recrystallisation the trianhydrogenin collects in the first crops. It may be purified by numerous recrystallisations, but is more readily purified by treatment with acetic anhydride. The crude crystals were warmed on the water-bath for 75 minutes with acetic anhydride (20 c.c.). On cooling, the nearly pure compound separated. It was recrystallised a few times from alcohol and from dilute pyridine until the specific rotation was constant. Trianhydroemicymarigenin crystallises in long, pale yellow needles which sinter at 190° and melt at 192°. The melting point varies somewhat with the rate of heating. It has $[\alpha]^{19°}_{5461}$ − 167·0°, $[\alpha]^{19°}_{D}$ − 138·5° (c = 2·45 in pyridine). The substance is sparingly soluble in the usual solvents, but more so in hot alcohol or ethyl acetate. It is readily soluble in pyridine. It dissolves in sulphuric acid to give a reddish-violet colour turning to red. In the Keller reaction a red ring is obtained, the top layer gradually assumes a pale green colour, and the bottom layer a red colour (Found: C, 82·1; H, 8·4; M, by lactone titration, 360. Calc. for $C_{23}H_{28}O_2$: C, 82·1; H, 8·4%; M, 336).

Trianhydroperiplogenin was prepared from the periplogenin from the easily hydrolysed glucosides by the method described by Jacobs and Bigelow (*J. Biol. Chem.*, 1933, **101**, 697), who after one recrystallisation obtained a product which melted at 191—193°, depending on the rate of heating, had $[\alpha]^{24°}_{5461}$ − 130° (in pyridine), and gave the correct analytical figures. After one recrystallisation our material sintered at 184° and melted indefinitely at 194° and had

$[\alpha]_D^{23°}$ − 118° (in pyridine). It was purified by treatment with acetic anhydride with subsequent crystallisation from alcohol as carried out in the case of trianhydroemicymarigenin. It separated from alcohol in thin, pale yellow, anhydrous needles which sintered at 187° and melted at 192°. $[\alpha]_{5461}^{23°}$ − 167·0°, $[\alpha]_D^{23°}$ − 136·8° (c = 1·76 in pyridine). The colour reaction with sulphuric acid was as described by Jacobs and Bigelow (Found: C, 81·9; H, 8·2. Calc. for $C_{23}H_{28}O_2$: C, 82·1; H, 8·4%). Trianhydroperiplogenin appears to be identical with trianhydroemicymarigenin. The specific rotations, melting points, and the sulphuric acid colour reaction were all similar and a mixture of the two showed no depression of the melting point. Both specimens had the same optical characters under polarised light. The crystals were optically biaxial and showed parallel extinction.

Anhydroemicymarigenin, $C_{23}H_{32}O_4$.—The alcoholic mother-liquors obtained above after removal of the crude crystalline trianhydro-compound were treated with water and the precipitate was dissolved in ethyl acetate. On standing, a small amount of crystals separated. They were recrystallised twice from methyl ethyl ketone. *Anhydroemicymarigenin* forms short white rods, m. p. 269—277°. It has $[\alpha]_{5461}^{24°}$ + 67°, $[\alpha]_D^{24°}$ + 59° (c = 3·0 in pyridine). It is sparingly soluble in the usual solvents except pyridine (Found: C, 74·1; H, 8·7. $C_{23}H_{32}O_4$ requires C, 74·1; H, 8·7%).

Digitalose, $C_7H_{14}O_5$.—Emicymarin (3·0 g.) was dissolved in warm alcohol (6 c.c.), water (184 c.c.) and 10% hydrochloric acid (10 c.c.) were added, and the solution was boiled under reflux for 2½ hours. The reducing value of the solution was then constant. The aglucone separated as an oil and was removed by extraction with chloroform. Yield, 1·8 g. The aqueous liquor was treated with silver carbonate to remove hydrochloric acid and then with hydrogen sulphide to remove the excess of silver. After evaporation and drying in a vacuum over sulphuric acid a syrup (0·75 g.) remained. It crystallised from a solution in dry ether on addition of light petroleum. From ethyl acetate it separated in rosettes of needles. The melting point rose when the solid was kept. Originally it sintered at 101° and melted at 106°. After 3 days it melted at 115° and after 4 months at 119°. The specific rotation rose from $[\alpha]_{5461}^{27°}$ + 109° (15 mins. after solution) to $[\alpha]_{5461}^{22°}$ + 126°, $[\alpha]_D$ + 106° (c = 1·7 in water) in 17 hours and was then constant. The reducing value of the sugar was determined by the Bertrand micro-method. Under these conditions 1 mg. had the same reducing value as 0·32 mg. of anhydrous glucose. The Keller reaction was negative. For analysis the sugar was dried at 70° in a vacuum (Found: C, 47·4; H, 7·9; OMe, 17·4. Calc. for $C_7H_{14}O_5$: C, 47·2; H, 7·9; OMe, 17·4%).

Digitalonolactone, $C_7H_{12}O_5$.—The sugar (368 mg.) in water (2·2 c.c.) was shaken with bromine (0·16 c.c.) for 1 hour. After standing over-night, the excess of bromine was removed in a vacuum by a current of air, the hydrobromic acid was removed with silver carbonate, and the excess of silver by hydrogen sulphide. The solution was evaporated on the steam-bath. The residue solidified in small rectangular crystals. After recrystallisation from alcohol it formed long white rhombic needles, m. p. 137—138°. $[\alpha]_{5461}^{19°}$ − 102°, $[\alpha]_D^{19°}$ − 83° (c = 3·23 in water). There was a slight fall in rotation when the solution was kept. For analysis the lactone was dried in a vacuum over phosphoric oxide. The lactone group was determined by boiling with excess of N/100-barium hydroxide for 15 minutes (Found: C, 48·0; H, 7·0; OMe, 17·2; M, by lactone titration, 179. Calc. for $C_7H_{12}O_5$: C, 47·7; H, 6·9; OMe, 17·6%; M, 176). For comparison, digitalonolactone was prepared from the mixed sugars from *digitalinum verum* by the method given by Kiliani (*Ber.*, 1930, **63**, 2868). It melted at 135—137° and, mixed with the above lactone, at 135—138°. It crystallised in rhombic needles. $[\alpha]_{5461}^{20°}$ − 102°, $[\alpha]_D^{20°}$ − 85° (c = 3·26 in water). Digitalonolactone was described by Kiliani (*Ber.*, 1892, **25**, 2117) as rhombic crystals having $[\alpha]_D^{28°}$ − 79·4° (c = 3·36). The melting point was not recorded.

Diacetylemicymarin.—The anhydrous glucoside (0·532 g.) was dissolved in a mixture of pyridine (5 c.c.) and acetic anhydride (2 c.c.). After several hours the mixture was diluted with water. The *acetate* separated in micro-plates. It crystallised from methyl alcohol in anhydrous plates, m. p. (indefinite) *ca.* 278°. $[\alpha]_{5461}^{20°}$ + 27·8°, $[\alpha]_D^{20°}$ + 22·8° (c = 1·4 in methyl alcohol) (Found: C, 64·0; H, 7·9; OMe, 5·0; $CH_3{\cdot}CO$, 13·9; M, by lactone titration, 210. $C_{34}H_{50}O_{11}$ requires C, 64·3; H, 7·9; OMe, 4·9; $CH_3{\cdot}CO$, 13·6%; M, 634).

Dihydroemicymarin.—The anhydrous glucoside (0·46 g.) in 95% alcohol (10 c.c.) was shaken with hydrogen and platinum oxide catalyst (0·5 g.). In 8½ hours 18·7 c.c. of hydrogen at $N.T.P.$ were absorbed (Calc. for one double bond, 18·7 c.c.). The alcoholic solution was concentrated and treated with water. The *dihydro-compound* separated in fine needles. The Legal reaction was negative (Found: H_2O, varying from 11·6 to 18·0% depending on the temperature of crystallisation. $C_{30}H_{48}O_9,4H_2O$ requires H_2O, 11·5%. $C_{30}H_{48}O_9,7H_2O$ requires H_2O, 18·6%).

The anhydrous substance sinters at 147° and melts indefinitely at 151°. It has $[\alpha]^{19°}_{5461}$ + 11·2°; $[\alpha]^{19°}_{D}$ + 8·6° (c = 6·4 in alcohol) (Found for material dried at 100° in a vacuum: C, 64·9; H, 8·8; OMe, 5·4. $C_{30}H_{46}O_9$ requires C, 65·2; H, 8·8; OMe, 5·6%).

iso*Emicymarin*.—Anhydrous emicymarin (2·2 g.) was dissolved by warming in methyl alcohol (25 c.c.); methyl alcohol (15 c.c.) containing 2·1 g. of caustic potash was added to the cooled solution. After standing at room temperature for 2 hours, the solution was poured into cold water and acidified (Congo-red) with hydrochloric acid. The iso*glucoside* separated slowly as a mass of fine needles. It contains two molecules of water, which it loses slowly on exposure to air. It is sparingly soluble in water or ethyl alcohol, but more so in methyl alcohol, from which it crystallises in the anhydrous form. It then sinters at 252° and melts at about 270° depending on the rate of heating. The anhydrous *iso*glucoside has $[\alpha]^{25°}_{5461}$ + 30·9°, $[\alpha]^{20°}_{D}$ + 26·2° (c = 1·7 in pyridine). The Legal reaction is negative (Found for the substance dried at 100° in a vacuum: C, 65·5; H, 8·3; OMe, 5·6; M, by lactone titration, 525. $C_{30}H_{46}O_9$ requires C, 65·4; H, 8·4; OMe, 5·0%; M, 550). *iso*Emicymarin was not reduced under the conditions which reduce the normal glucoside. Hydrolysis by boiling 50% alcohol containing 5% of hydrochloric acid gave a small yield of an aglucone, $C_{23}H_{34}O_5$, resembling *iso*periplogenin (Jacobs and Hoffmann, *J. Biol. Chem.*, 1928, **79**, 529). The quantity obtained was too small to make the identification certain. Digitalose also was isolated.

The crystalline water-soluble glucoside separated from hot water in sheaths of white micro-needles, m. p. 205—207°. The material dried at 115° in a vacuum had $[\alpha]^{21°}_{5461}$ − 2·0°, $[\alpha]^{21°}_{D}$ − 1·7° (c = 5·0 in 95% alcohol) (Found: C, 60·5; H, 8·2; OMe, 4·7; CH_3·CO, 1·6. Calc. for $C_{36}H_{55}O_{14}$·CO·CH_3: C, 60·3; H, 7·7; OMe, 4·1; CH_3·CO, 5·7%). The low acetyl figure indicates that the substance is a mixture. Hydrolysis by 0·5% hydrochloric acid at 95° gave a syrup which appeared to be a biose. This was further hydrolysed by 5% sulphuric acid at 100°. From the mixture of sugars obtained, glucosazone was isolated; its identity was confirmed by the mixed melting point (Found: N, 15·8. Calc. for glucosazone: N, 15·6%). Digitalose also was isolated.

Allomerisation Process.—The fat-free seeds (1 kg.) were mixed with water (4 l.) and a little toluene and left at about 25° for 14 days. The mixture was treated with alcohol (15 l.) and filtered. The solution was purified with basic lead acetate, freed from lead, and concentrated. During the evaporation the sparingly soluble *allo*glucosides separated as a pasty solid, which was collected, extracted with chloroform, and crystallised once from dilute alcohol. It then weighed 3·6 g. The original mother-liquor after removal of the *allo*glucosides was extracted with chloroform. This extract was combined with the chloroform extract of the solid and evaporated. The residue (31·5 g.) was dissolved in methyl alcohol; 8·4 g. of crude cymarin then separated. When the methyl-alcoholic mother-liquor was treated with water, 8·7 g. of crude *allo*glucosides were precipitated. The filtrate contained emicymarin.

The final liquor after removal of the chloroform-soluble glucosides yielded 6·8 g. of water-soluble amorphous glucosides. These were dissolved in water, and the solution partially saturated with ammonium sulphate. A small amount of the bioside of Jacobs and Bigelow separated.

The crude *allo*glucosides were fractionally crystallised first from dilute alcohol and then from ethyl acetate and acetone. The less soluble material contained a mixture of at least two glucosides, which have not been separated. It resembled *allo*cymarin and possibly was a mixture of *allo*cymarin and *allo*periplocymarin. From the mother-liquors was obtained a monoside identical with that of Jacobs and Bigelow. It was purified by crystallisation first from dilute alcohol, then from ethyl acetate, and finally from methyl alcohol. It crystallises from dilute alcohol as a mass of micro-needles, from methyl alcohol in small plates or leaflets, and from ethyl acetate in rectangular plates. It is readily soluble in methyl and ethyl alcohols and sparingly soluble in acetone and ethyl acetate. The anhydrous glucoside sinters at 240° and melts at 248°. After crystallisation from dilute alcohol it contains 9% of water and then sinters at 170° and melts at 263°. The anhydrous material has $[\alpha]^{25°}_{5461}$ + 29·7°, $[\alpha]^{23°}_{D}$ + 24·6° (c = 1·85 in 95% alcohol). Jacobs and Bigelow's monoside melted at 174—180° and had $[\alpha]^{28°}_{D}$ + 22° (c = 0·995 in 95% alcohol). It gives a positive Legal reaction. The Keller reaction is practically negative. Dissolved in sulphuric acid, it gives a reddish-brown solution. The taste is slightly bitter (Found for the substance dried at 110° in a vacuum: C, 65·0; H, 8·4; OMe, 6·1. Calc. for $C_{30}H_{46}O_9$: C, 65·4; H, 8·4; OMe, 5·6%).

Hydrolysis of allo*Emicymarin*.—The anhydrous glucoside (2·73 g.) in 30% alcohol (158 c.c.) containing 0·5% of hydrochloric acid was heated in the steam-bath for 3½ hours. The aglucone separated as a resin (1·95 g.) which could not be crystallised. It was converted into the anhydro-derivatives by boiling under reflux for 2 hours with methyl alcohol (25 c.c.) containing 6% of

hydrogen chloride. The solution was treated with excess of water, and the precipitate fractionally crystallised first from alcohol and then from ethyl acetate. The trianhydrogenin separated first. The anhydrogenin was obtained from the mother-liquors. The dilute alcoholic liquor obtained during the original hydrolysis was extracted with chloroform to remove traces of the aglucone and the sugar was isolated by the method used in the case of emicymarin. It was identified as digitalose and had the properties described above.

Trianhydro*allo*emicymarin separated from alcohol in faintly yellow leaflets or irregular shaped plates, m. p. 153—156°. $[\alpha]^{23°}_{5461}$ — 106·4°, $[\alpha]^{23°}_{D}$ — 83·5° (c = 3·07 in pyridine) (Found : C, 81·9; H, 8·5. Calc. for $C_{23}H_{28}O_2$: C, 82·1; H, 8·4%). Jacobs and Bigelow give $[\alpha]^{27°}_{D}$ — 84·6° (c = 1·015 in pyridine) and m. p. 154—156°.

*Anhydro*allo*emicymarin* crystallised from ethyl acetate in nearly colourless needles or rectangular plates, m. p. 208—209°. $[\alpha]^{22°}_{5461}$ + 157°, $[\alpha]^{22°}_{D}$ + 133° (c = 4·77 in pyridine). It dissolved in sulphuric acid to give a yellow-orange solution. In the Keller reaction the top layer was dull green and the bottom layer brown. The substance was anhydrous (Found : C, 74·2; H, 8·7. $C_{23}H_{32}O_4$ requires C, 74·1; H, 8·7%).

Conversion of Emicymarin into allo*Emicymarin.*—The enzyme-containing marc was obtained by extracting powdered defatted *S. Emini* seeds twice with ice-cold water and then further by percolation with 95% alcohol until nearly free from glucosides. Emicymarin methyl alcoholate (0·5 g.) was dissolved in water (150 c.c.) by warming. The strophanthus marc (40 g.) was added together with a few c.c. of toluene, and the mixture left at room temperature for 55 days. Since the marc still contained a trace of glucoside, a duplicate experiment was done without the emicymarin. Both lots were worked up by the method given above under allomerisation. The yields of the crude fractions were :

		Blank.
*allo*Glucosides	0·38 g.	<0·01 g.
Chloroform-soluble glucosides	0·14 g.	0·16 g.
Water-soluble glucosides	0·04 g.	0·05 g.

The *allo*glucosides were crystallised from hot ethyl acetate and gave colourless plates (0·31 g.), m. p. 250—258°. The anhydrous material had $[\alpha]^{22°}_{5461}$ + 28·9°, $[\alpha]^{22°}_{D}$ + 24·0° (c = 2·0 in 95% alcohol) (Found after drying at 120° in a vacuum : C, 64·9; H, 8·4; OMe, 5·7. Calc. for $C_{30}H_{46}O_9$: C, 65·4; H, 8·4; OMe, 5·6%). When hydrolysed at 100° by methyl alcohol containing 6% of hydrogen chloride, the glucoside yielded a small amount of an anhydrogenin, m. p. 207°. This was identical in appearance and reactions with the anhydrogenin from *allo*emicymarin and a mixture of the two melted at 208° (Found : C, 74·6; H, 9·0. Calc. for $C_{23}H_{32}O_4$: C, 74·1; H, 8·7%).

Glucose from E. Strophanthin.—The isolation was difficult, since on acid hydrolysis a mixture of sugars was obtained. It was expected that enzyme hydrolysis of the total glucosides would, after removal of the glucosides, give a solution containing glucose, but the presence of impurities prevented its isolation. The solution, however, yielded an osazone which was identified as glucosazone by its appearance, m. p. and mixed m. p. The rotation of the osazone was determined. 0·15 G. in 5 c.c. of pyridine and 10 c.c. of alcohol in a 2 dcm. tube had an initial rotation $\alpha^{23°}_{5461}$ — 1·73°. After 24 hours the value had dropped to — 0·98°. Under the same conditions pure glucosazone gave the values — 1·71° and — 0·91° respectively.

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