LXXXV.—*Glycyphyllin, the Sweet Principle of Smilax glycyphylla.*

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In a paper by Dr. C. R. Alder-Wright and the author which appeared in the Journal of this Society (Trans., 1881, 237), a partial description was given of a crystalline substance extracted from the leaves of *Smilax glycyphylla*,* a plant growing in abundance on the shores of Port Jackson, and common on the coasts of the northern parts of New South Wales and the southern parts of Queensland. The investigation was not completed from want of material, and although the writer obtained a grant from the Society some three years ago for a further examination of this substance, other duties have prevented, until quite recently, the completion of the work.

The results obtained confirm in some respects those published in the paper above referred to, but have shown that the formula therein proposed needs alteration. In brief, the substance has the formula $C_{21}H_{24}O_9 + 3H_2O$ when crystallised from aqueous ether, and $C_{21}H_{24}O_9 + 4\frac{1}{2}H_2O$ when crystallised from water. When boiled with dilute sulphuric acid, it undergoes decomposition in accordance with the equation—

forming *phloretin* and *isodulcite*, and is therefore closely allied to phlorizin. A discussion of these results will be found in the detailed account which follows.

Extraction and Purification.

In order to obtain the material for this investigation, about 75 lbs. weight of the leaves and stems were macerated for several days with strong spirit (till the leaves became tasteless) and the alcohol distilled off. The dark-coloured syrupy liquid was then exhausted with ether (a tedious process, as ether does not dissolve the substance very

* As the leaves treated were not submitted to an expert, it is possible that among them were some from *Smilax australis*, but the majority were from the above-mentioned species.

freely), and the ether distilled off. The residue always solidified on cooling to a dark-brown or yellow mass of crystals mixed with more or less semi-fluid resinous matter. The best method of effecting a rough purification was found to be solution and repeated crystallisation from hot water containing a *little* alcohol, rejecting any resinous matter which refused to dissolve easily. By this means, the greater part of the colouring matter could be removed and a mass of yellowish crystals was obtained, which, however, obstinately refused to become colourless by crystallisation alone. The further purification was effected by one of two methods.

1. The substance was dissolved in warm water, allowed to cool until just about to crystallise, then quickly shaken up with ether, and the latter rapidly poured off through a dry filter into a dry flask. On standing, a great part of the substance separated out as a perfectly white mass of minute crystals.

2. The substance was dissolved in hot water, some acetate of lead added, and the precipitate, which formed in larger or smaller quantity according as the solution was darker or lighter in colour, filtered through a hot-water filter and rejected. Sulphuretted hydrogen was then passed through the solution, the lead sulphide filtered off, the liquid being still warm enough to keep the substance in solution, and the filtrate allowed to crystallise. It was sometimes found necessary to repeat this process in order to get a perfectly white product, but it was finally adopted as the easier.

Composition and Properties.

The crystals deposited from a warm aqueous solution consisted of very brittle, slender four-sided prisms, often from one-half to one inch in length, if the solution had been slowly cooled. Whether separated from ether (under the conditions described above) or from water, they contain water of crystallisation, but not the same in amount (details given below). The following are the results of several combustions of different samples of anhydrous substance (dried at from 100—110°), Nos. I, II, III prepared by the ether process, and the remainder by the acetate of lead process:—

	Substance.	CO ₂ .	H ₂ O.
I	0.3535	0.7735	0.1980
II	0.2680	0.5865	0.1425
III	0.3073	0.6689	0.1640
IV	0.3390	0.7375	0.1825
V	0.3255	0.7100	0.1755
VI	0.3249	0.7085	0.1735
VII	0.2902	0.6352	0.1550
VIII	0.2474	0.5404	0.1314

Calculated for $C_{21}H_{24}O_9$		I.	II.	III.	IV.
C_{21}	252 60·00	59·67	59·35	59·36	59·33
H_{24}	24 5·70	6·22	5·83	5·93	5·98
O_9	144 34·30	—	—	—	—
	420 100·00				

	V.	VI.	VII.	VIII.
C	59·49	59·47	59·69	59·57
H	5·99	5·93	5·93	5·90
O	—	—	—	—

The following table gives in percentages the results of several water determinations made in *different* samples, dried at 100—110°:—

	Mean.					
Crystallised from ether	11·31	11·88	11·84	11·43	11·13	11·54
Crystallised from water	16·28	16·17	16·31	16·11	—	16·22

The formula $C_{21}H_{24}O_9 + 3H_2O$ requires 11·39 per cent. of water.

„ $C_{21}H_{24}O_9 + 4\frac{1}{2}H_2O$ „ 16·16 „ „

A combustion of an *air-dried* specimen (crystallised from water) gave carbon 49·85 per cent., hydrogen 6·55 per cent., theory requiring carbon 50·29 per cent., and hydrogen 6·58 per cent., thus confirming the above results.

For the present at least, I propose to retain for this substance the name *glycophyllin*, suggested in the joint paper already quoted, as it appears to be quite different from the substance described under the name of *smilacin* and from other known substances. It has no well-defined melting point, but at temperatures varying from about 110° to 115° it begins to show signs of change, and as the temperature rises, is gradually transformed into a caramel-like substance, which at 175—180° melts with decomposition. It is only very sparingly soluble in cold water, but sufficiently so to communicate to the solution its characteristic strong liquorice-like taste. It is easily soluble in hot water and alcohol, soluble to some extent in ether, insoluble in chloroform, benzene, and light petroleum. It dissolves in solutions of the caustic alkalis, and the solution gradually assumes a rich reddish-brown colour on exposure to the air. It does not reduce Fehling's solution. Its solution is not precipitated by ordinary acetate of lead, but is immediately precipitated by the basic acetate.

It was not possible in this case to get an accurate estimate of the yield of material, but it could not have been more than one-half, and was probably nearer one-third per cent.

Decomposition by Dilute Sulphuric Acid.

When an aqueous solution of glycyphyllin is boiled in a flask with reversed condenser, with the addition of a small quantity of dilute sulphuric acid, the liquid soon becomes turbid, and rapidly deposits an almost colourless crystalline powder. On cooling, a small quantity more separates out, the liquid having at the same time acquired the power of reducing Fehling's solution. As the precipitated substance is never obtained quite white, it is purified by dissolving it in a hot mixture of alcohol and water (about equal volumes), adding acetate of lead, filtering off any slight precipitate, passing sulphuretted hydrogen, &c., &c. The filtrate, on cooling, deposits a snow-white crystalline mass, which is anhydrous. The following are the analytical numbers obtained from different samples:—

	Substance.	CO ₂ .	H ₂ O.
I	0.2030	0.4857	0.1015
II	0.2690	0.6486	0.1295
III	0.2885	0.6962	0.1370
IV	0.2558	0.6170	0.1220
V	0.2535	0.6063	0.1225
VI	0.2532	0.6064	0.1244

	Calculated for C ₁₅ H ₁₄ O ₅ .		I.	II.	III.	IV.	V.	VI.
C ₁₅ ..	180	65.69	65.25	65.76*	65.82*	65.78*	65.22	65.31
H ₁₄ ..	14	5.11	5.52	5.35	5.28	5.29	5.36	5.45
O ₅ ..	80	29.20	—	—	—	—	—	—
	274	100.00						

These numbers agree satisfactorily with those required by the formula C₁₅H₁₄O₅, which is ascribed to *phloretin*, and, on further examination, leaves no doubt as to the identity of the two substances. The substance obtained as described above is almost insoluble in cold water, and only very sparingly soluble in boiling water, from which it crystallises on cooling in minute shining prisms. It is easily soluble in alcohol, and crystallises from a hot mixture of alcohol and water in groups of needles radiating from a centre. It is soluble also in ether and hot glacial acetic acid. It melts at 250° with decomposition. Stas gives the melting point of *phloretin* as 180°, and this at first misled me, but it has since been shown by Schiff (*Ber.*, 14,

* In these combustions, the oxygen which had been used in the combustions was inadvertently allowed to remain in the tubes while weighing, thus accounting to some extent for a somewhat high percentage of carbon.

303) that the melting point is close to 250° . It dissolves with yellow coloration in solutions of the caustic alkalis and of the alkaline carbonates, and is reprecipitated on acidifying. The solution in strong ammonia, after a short time, deposits golden-yellow scales. When boiled with strong caustic potash for from 15 to 20 minutes, the solution is no longer precipitated on addition of an acid, and is found to contain *phloroglucol* and an acid which is to all appearance identical with *phloretic acid*.

To isolate these substances, the alkaline solution was first slightly acidified with hydrochloric acid, rendered slightly alkaline with sodium hydrogen carbonate, and then exhausted with ether. The residue after the evaporation of the ether was decolorised partly by the aid of animal charcoal and partly by the acetate of lead process, and then crystallised several times from water. The crystals contained water of crystallisation, and had a very sweet taste. The melting point of the anhydrous substance was found to be $208-209^{\circ}$.* The aqueous solution gave a deep reddish colour with ferric chloride. On combustion, the following numbers were obtained:—

	Substance.	CO ₂ .	H ₂ O lost.
I	0.2220	0.4627	—
II	0.3046	0.6328	0.1357
	Calculated for C ₆ H ₆ O ₃ .		
	<div style="text-align: center;">┌───────────┐</div>		
C ₆	72	57.14	I. 56.84
H ₆	6	4.76	— 4.95
O ₃	48	38.10	— —
	126	100.00	

There can be no doubt, therefore, that the substance in hand was *phloroglucol*.

The solution from which the *phloroglucol* had been removed was acidified by hydrochloric acid, and again exhausted with ether. On evaporation, a mass of dark-coloured prismatic crystals remained. These were decolorised by animal charcoal and several times recrystallised from hot water; by slow cooling, crystals were often obtained an inch in length. On combustion, the following numbers were obtained:—

	Substance.	CO ₂ .	H ₂ O.
I	0.2532	0.6000	0.1440

* This is the melting point given in all the more recent accounts of *phloroglucol*, although in *Miller's Chemistry* and elsewhere it is stated to be about 220° .

	Calculated for $C_9H_{10}O_3$.		I.
C_9	108	65.09	64.63
H_{10}	10	6.03	6.32
O_3	48	28.88	—
	166	100.00	

The silver salt was prepared by precipitating a solution of silver nitrate with a solution of the barium salt (obtained by boiling the acid with pure barium carbonate), washing the precipitate in a partially darkened room, drying it in the dark over sulphuric acid, and finally at 100° . It gave the following numbers on analysis:—

	Substance.	CO_2 .	H_2O .	Ag.
I	0.3086	0.4494	0.0995	0.1212

	Calculated for $C_9H_9AgO_3$.		I.
C_9	108	39.56	39.72
H_9	9	3.58	3.29
Ag	108	39.56	39.27
O_3	48	17.30	—
	273	100.00	

The acid melted at 127 — 128° , and its aqueous solution gave a *slight* violet coloration with ferric chloride. In other physical characteristics, it agreed closely with the description given of phloretic acid, and it is obviously identical with the acid previously obtained by fusing glycyphyllin with caustic potash at 250° . Trinius, who has synthesised phloretic acid (*Annalen*, **227**, 262), states that when pure it gives no coloration with ferric chloride, but says that a sample prepared from phlorizin gave this coloration, and he concludes that the reaction is due to a small quantity of impurity in the acid, and that Rochleder's so-called isophloretic acid was really pure phloretic acid. The acid prepared as above described, even when repeatedly crystallised from water, converted into the silver salt, and regenerated from the latter, still gave the reaction. The colour produced, however, could hardly be said to be well marked, and is probably due, as Trinius suggests, to the presence of some impurity (phloroglucol?) which obstinately adheres to the acid prepared in this way. It may be remarked, however, that a single drop of ferric chloride solution, such as is usually employed as a reagent, gives scarcely a perceptible coloration, but the colour deepens considerably

on the addition of a further quantity. This may perhaps account to some extent for the discrepancy.

Examination of the Filtrate after Separation of the Phloretin.

The filtrate from the phloretin was neutralised by the addition of barium carbonate, the barium sulphate and excess of carbonate filtered off, and the solution evaporated to dryness. During this process, more or less of a red colouring matter was produced, but the syrup which remained soon crystallised. The crystals were washed once or twice with a little cold strong alcohol which removed most of the colouring matter, and then repeatedly crystallised from ordinary alcohol, but the product so obtained always had a slight molasses-like odour, and on combustion gave numbers which did not agree well with any probable formula. It was, therefore, recrystallised two or three times from very strong alcohol ($98\frac{1}{2}$ per cent.) until quite white and free from odour. Specimens so prepared melted at $93-94^\circ$ when slowly heated in a capillary tube. It gave up one molecule of water on being heated at 100° . Three different samples gave the following results on analysis:—

	Substance.	CO ₂ .	H ₂ O.
I	0.2513	0.3657	0.1800
II	0.2495	0.3609	0.1785
III	0.2660	0.3828	0.1849

	Calculated for C ₆ H ₁₄ O ₆ .		I.	II.	III.
C ₆	72	39.55	39.68	39.45	39.25
H ₁₄	14	7.69	7.95	7.95	7.72
O ₆	96	52.76	—	—	—
	182	100.00			

This substance was found to be sparingly soluble in cold alcohol, but easily soluble in the boiling liquid, from which, or from water by slow evaporation, it separated in well-formed monoclinic crystals agreeing exactly in appearance with the crystals of isodulcite obtained by Will from *naringin*, and figured by him (*Ber.*, **18**, 1318). It reduced Fehling's solution on boiling. The quantity obtained was insufficient to determine its specific rotatory power, but the above evidence is, I think, sufficient to identify it as isodulcite.

Quantitative Examination of the Decomposition by Dilute Sulphuric Acid.

In order to determine the equation representing the decomposition of glycyphyllin by dilute sulphuric acid, weighed quantities were boiled with suitable proportions of the dilute acid in a flask connected with a reflux condenser until the decomposition was judged to be complete. The liquid was allowed to cool, the phloretin filtered off, well washed with cold water, washed off the filter with alcohol, and the alcoholic solution evaporated to dryness. The filtrate and washings were then extracted with ether to remove small quantities of phloretin which still remained in solution, and the residue from the evaporation of the ether added to the main portion, the total product being finally dried at 100–110°. Four experiments of this kind were made with the following results:—

	Glycyphyllin (anhydrous).	Phloretin.	Per cent.
I	1·224	0·8055	65·63
II	11·479	7·463	65·01
III	5·239	3·474	66·31
IV	5·0033	3·317	66·30

In the last of these experiments, the liquid freed from phloretin was made up to 1 litre, and titrated with Fehling's solution. It was found that 26 c.c. were required to reduce 10 c.c. of Fehling's solution (1 c.c. = 0·005 glucose). Assuming that 10 c.c. Fehling's solution are reduced by 0·0549 gram isodulcite,* as recently determined by Will (*loc. cit.*), we have—

$$\text{total isodulcite} = \frac{1000 \times 0\cdot0549}{26} = 2\cdot115 \text{ grams.}$$

This is equal to 42·2 per cent. on the glycyphyllin taken. Considering the formation of small quantities of secondary products, such as colouring matter, these numbers agree as well as can be expected with those indicated by the equation—

which requires 65·22 per cent. of phloretin and 43·33 per cent. of isodulcite.

Discussion of above Results.

The formation of isodulcite by the decomposition of glycyphyllin

* In an actual experiment, a number very close to this was obtained for the reducing power of isodulcite, but the details and therefore the exact numbers were unfortunately lost.

is interesting as affording another instance of a so-called glucoside which yields isodulcite and not glucose on boiling with dilute acids. It has been shown that isodulcite has more than once been mistaken for glucose (*Ber.*, 15, 215; 18, 1316, *et seq.*). During the course of this investigation, it seemed possible that this might have happened in the examination of phlorizin, in which case glycyphyllin might be identical with that substance. The close resemblance in physical properties seemed to render this probable. Thus Schiff (*Ber.*, 14, 303) states that phlorizin "melts at 110° to a semi-fluid opaque mass, which on further heating became again solid and porcelain-like, and at 170—171° melts to a clear yellow fluid;" this is very like the behaviour of glycyphyllin. Schiff also calls attention to Loewe's criticism on the results published with reference to phlorizin, especially as regards the discrepancies in the analytical numbers obtained.

On closer examination, however, the differences appear to be too great to be accounted for by any experimental error. The highest percentage of carbon obtained in the analysis of phlorizin is about 2 per cent. lower than that required for glycyphyllin; the amount of water of crystallisation found in phlorizin by several experimenters corresponds with only 2 mols. H_2O ; and, finally, although Hesse states (*Miller's Organic Chemistry*, 5th edition, p. 582) that the sugar obtained from phlorizin is essentially different from glucose, yet he finds for its specific rotatory power in a solution freshly prepared $[\alpha]_D = 89$, a number more than 10 times as great as that given by several observers for isodulcite.

I have sent to Trommsdorf for a supply of phlorizin, in order to compare it with glycyphyllin, and if possible to ascertain the relationship between these two substances, and I hope, ere long, to communicate the results to the Society.