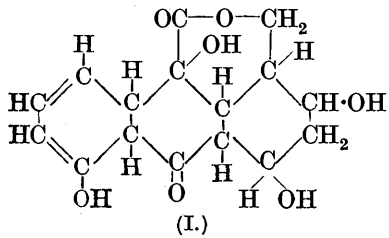
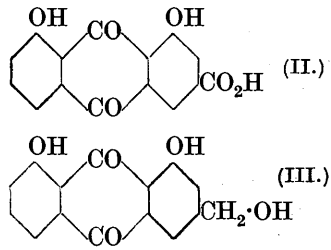
LXXVIII.—*Experiments on the Constitution of the Aloins. Part II.*

By CHARLES STANLEY GIBSON and JOHN LIONEL SIMONSEN.

SOME years ago Robinson and Simonsen (J., 1909, **95**, 1085) suggested tentatively for barbaloin the formula (I), based chiefly on the following facts: (i) the careful investigations of Jowett and Potter (J., 1905, **87**, 878) had shown that barbaloin has the composition $C_{16}H_{18}O_7$ and contains four hydroxyl groups; (ii) the oxidation of acetylbarbaloin with chromic acid yields a mixture of acetylrhein and acetylaloe-emodin; (iii) rhein was shown to be a dihydroxyanthraquinonecarboxylic acid, and aloe-emodin the corresponding primary alcohol. At the time, it was considered probable that rhein was an *iso*chrysazincarboxylic acid, but the further work of Robinson and Simonsen, in the publication of the results of which they were anticipated by other workers (Oesterle and co-workers, *Arch. Pharm.*, 1909, **247**, 417, 532; 1911, **250**, 305), showed that rhein (II) and aloe-emodin (III) are derivatives of chrysazin.



(I.)

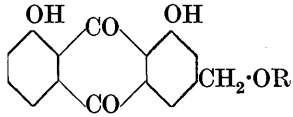


(II.)

(III.)

This conclusion obviously necessitated a revision of the suggested constitution for barbaloin and it has only recently been possible to resume the investigation. In the meantime, Léger (*inter alia*, *Ann. Chim.*, 1916, **6**, 318; 1917, **8**, 265) has published the results of a prolonged investigation of the constitution of barbaloin and of other members of this group. He concludes that the composition of barbaloin is not $C_{16}H_{18}O_7$ with four hydroxyl groups but $C_{20}H_{18}O_9$ having five hydroxyl groups. Elementary analysis of either the parent substance or of certain of its simple derivatives does not distinguish between these two formulæ, and Léger was led to make the change by the important observation that when barbaloin is treated with alcoholic hydrochloric acid under specified conditions *d*-arabinose is formed in addition to aloe-emodin. He naturally concluded that barbaloin is the arabinoside of aloe-emodin

having the constitution (IV), although the actual position of attachment of the sugar residue was not definitely proved.



(IV.)

R = *d*-arabinose.

The suggestion that barbaloin was a glucoside was not new, since Rochleder and Czumpelik (*Monatsh.*, 1863, **44**, 493; 1866, **47**, 119) had observed the formation of a sugar in addition to a yellow crystalline substance by the action of alcoholic hydrochloric acid on barbaloin: Tschirch and Pedersen (*Arch. Pharm.*, 1898, **236**, 206), however, were unable to confirm this. Léger conclusively proved that *d*-arabinose is formed when barbaloin is kept with alcoholic hydrochloric acid for a period of six months, and we have confirmed this. The rate of hydrolysis, if such it be, is extraordinarily slow and very little action appears to have taken place after a week's continuous digestion, although the barbaloin is completely in solution. Léger also observed that aloe-emodin and *d*-arabinose are produced when barbaloin is oxidised with sodium peroxide. In this reaction the sodium peroxide must be assumed to act as a hydrolytic agent, although the yield of aloe-emodin does not exceed 9%.

We have confirmed in all respects Léger's excellent experimental work, but it appears to us that there are a number of cogent reasons for doubting the correctness of the constitution he assigned to barbaloin. The more important of these may be summarised. As already mentioned, elementary analysis does not readily distinguish between the two formulæ which have been suggested, and if the tendency for barbaloin to retain solvent of crystallisation be taken into consideration the differences between the carbon and hydrogen contents lie within the limits of experimental error ($C_{16}H_{18}O_7$ requires C, 59·6; H, 5·6%. $C_{20}H_{18}O_9$ requires C, 59·7; H, 4·6%). Molecular-weight determinations have led to contradictory results. By the cryoscopic method, using phenol as the solvent, Jowett and Potter obtained the value 310. On the other hand, Seel and Kelber (*Ber.*, 1916, **49**, 2364) obtained the abnormal value of 197 with phenol; by the ebullioscopic method, however, they obtained the average values of 408 with acetone and 440 with ethyl alcohol. Although the last-mentioned results support the C_{20} formula, the analytical data for the chloro- and bromo-barbaloins, which are highly crystalline and readily purified substances, agree much more closely with the C_{16} formula; this is seen from the following

analytical results from the papers of Jowett and Potter and of Léger:

Chlorobarbaloin.	% C.	% H.	% Cl.	% Br.
Found	45·9	3·4	24·9, 25·1	
Calc. for $C_{16}H_{15}O_7Cl_3$	45·3	3·1	25·1	
Calc. for $C_{20}H_{14}O_9Cl_4$	44·4	2·6	26·3	
Bromobarbaloin.				
Found	34·1	2·7		42·9, 41·8, 43·1
Calc. for $C_{16}H_{15}O_7Br_3$	34·3	2·7		42·9
Calc. for $C_{20}H_{14}O_9Br_4$	33·4	1·9		44·6

Jowett and Potter determined the molecular weight of the bromo-compound in phenol by the cryoscopic method and obtained the value 535 ($C_{16}H_{15}O_7Br_3$ requires M, 559).

We have obtained analytical results for the bromo-compound (Found: Br, 42·6%) in agreement with those of previous investigators: the compound can be recrystallised without change from pyridine and digested for a short time with diethylaniline; further, its acetyl derivative both before and after treatment with sodium acetate in alcoholic solution has the same composition agreeing with the C_{16} formula. We are unable, therefore, to accept Léger's suggestion that the marked deficiency in halogen content for the C_{20} formula may be explained by the instability of the bromo- and chloro-compounds and their tendency to lose hydrogen halides.

Even more remarkable, in our opinion, is the resistance which barbaloin as a simple arabinoside shows to hydrolytic agents, a property which is shared by bromobarbaloin. The latter can be heated in a sealed tube at 100° with alcoholic sulphuric acid (20% by weight) without change. Again, barbaloin and its halogen derivatives dissolve in alkali, yielding bright yellow solutions: these would not be expected if the parent substance were a dihydroxyanthraquinone glucoside with two free phenolic hydroxyl groups, since such substances give red solutions.

We were therefore led to take up again the study of the chemistry of barbaloin and the results so far obtained appear to indicate that Léger's formula cannot be correct.

The action of reducing agents on barbaloin was first investigated, but with unsatisfactory results. The alcohol cannot be reduced with hydrogen in the presence of platinum oxide,* and on treatment with sodium and alcohol or with hydriodic acid and phosphorus it yields bright red products resembling the phlobaphenes in properties. Acetylation–reduction yielded a colourless gum which could not be purified.

Results of a greater interest have been obtained from a detailed study of the bromination of barbaloin. It has long been known

* We are indebted to Dr. H. King for carrying out this experiment.

that barbaloin on bromination with bromine water yields a bromo-barbaloin which is either tribromo- or tetrabromo-barbaloin according as the C_{16} or the C_{20} formula be adopted. Léger prepared a second bromo-compound, by bromination in hydrobromic acid solution, which differed completely in its properties from the ordinary bromobarbaloin and was considered by him to be the tribromide having the formula $C_{20}H_{15}O_9Br_3$. We have prepared this bromo-compound and confirmed its composition, but, on acetylation, contrary to expectation, it yields an amorphous *hexa-acetyl* and not a penta-acetyl derivative. The presence of six hydroxyl groups in this bromo-compound has been confirmed by the preparation of a *pentamethyl* ether by methylation with methyl sulphate, and as this ether can be acetylated it still contains a hydroxyl group. The analytical data (see p. 559) appear to indicate also that a methyl group is introduced into the nucleus, and the pentamethyl ether may therefore be formulated $C_{20}H_8O_3Br_3Me(OH)(OMe)_5$. The introduction of a methyl group into the nucleus does not require comment at this stage, since it is not infrequently observed when methyl sulphate is employed as a methylating agent.

These results rendered necessary the careful investigation of ordinary bromobarbaloin, which according to Léger is a tetrabromo-derivative. The difference in physical properties of the two bromo-compounds is very marked : the tribromo-compound is very sparingly soluble and microcrystalline, whereas the tetrabromo-compound is readily soluble and highly crystalline. This differentiation is much greater than that usually observed between tri- and tetra-bromo-derivatives. Our confirmation of the analytical results of previous workers for ordinary bromobarbaloin has been supplemented by molecular-weight determinations kindly carried out by Dr. Sugden by a new ebullioscopic method which he will describe. With acetone as the solvent, the molecular weight was found to be 538, in fair agreement with the calculated value 559 (C_{16} formula). When the bromo-compound is acetylated with acetyl chloride, a tetra-acetyl derivative, which has been previously described, is obtained, but if acetic anhydride is employed a different *acetyl* derivative is formed. Although this is amorphous, the analytical data show conclusively that it must contain five acetyl groups. The presence of five hydroxyl groups in this bromo-compound was confirmed by the preparation of a *pentamethyl* ether. This, like the acetyl derivative, is amorphous, but the analytical results indicate clearly that its composition is $C_{16}H_{10}O_2Br_3(OMe)_5$.

The result of the investigation of the bromo-compounds leads to the apparently anomalous position that, whereas by bromination in aqueous solution derivatives of a substance having the composition

$C_{16}H_{18}O_7$ are obtained, bromination in the presence of hydrobromic acid yields derivatives of a substance having the composition $C_{20}H_{18}O_9$. It seems to us that these results can be reconciled only by assuming that the bromo-compound prepared in hydrobromic acid is a true derivative of barbaloin. As suggested by Léger, barbaloin has, therefore, the composition $C_{20}H_{18}O_9$, but it contains not five but six hydroxyl groups, so the formula may be written as $C_{20}H_{12}O_3(OH)_6$ and that of the tribromo-compound as $C_{20}H_9O_3Br_3(OH)_6$.

With regard to the bromo-compound prepared in aqueous solution we suggest that it is an *oxidation product* formed by the loss of four carbon and two oxygen atoms and that it has the composition $C_{16}H_{10}O_2Br_3(OH)_5$. The methods adopted for its preparation (by the action of bromine water) and for the preparation of the analogous chloro-compound * (potassium chlorate and hydrochloric acid or chlorine in hydrochloric acid) are such as would readily permit of oxidation occurring simultaneously with halogenation, and the yields are very poor in both cases. The suggestion that the lower-melting bromo-compound is not a normal bromination product of barbaloin is supported indirectly by a number of comparative experiments which we have carried out. We have been unable to prepare the bromo-compound except by the use of bromine water; bromination of barbaloin in acetic acid solution containing sodium acetate yields only the sparingly soluble bromo-compound, $C_{20}H_{15}O_9Br_3$, and in chloroform solution a substance resembling this in properties, but having a somewhat higher bromine content, is obtained. The last substance requires further study, but we are of the opinion that it is probably a tetrabromo-derivative.

To facilitate differentiation between these two bromo-derivatives, we suggest that the higher-melting compound, $C_{20}H_{15}O_9Br_3$, should be known as *tribromobarbaloin*, and the lower-melting derivative as *tribromonorbarbaloin*.

The only evidence which we have discovered directly at variance with the suggestion now made is the observation of Léger that tribromonorbarbaloin and trichloronorbarbaloin yield tetrabromo- and tetrachloro-aloe-emodin, respectively, on oxidation with sodium peroxide in alkaline solution. This reaction needs further investigation: it probably involves more complex changes than the simple hydrolysis required by Léger's formula. This view is supported by the fact that we have not been able to oxidise either acetyltribromobarbaloin or acetyltribromonorbarbaloin to an anthraquinone derivative with chromic acid.

* We have prepared this chloro-compound and its composition is $C_{16}H_{15}O_7Cl_3$.

It is difficult to determine how far the results of the molecular-weight determinations which Dr. Sugden has kindly carried out on tribromonorbarbaloin pentamethyl ether bear on the present problem. These gave the average value of 844, the calculated molecular weight being 629. As has been mentioned, the substance is amorphous and separates from its acetone solution as a gum; the results of the molecular-weight determinations, however, are very concordant. For the present, we are inclined to regard the results as abnormal and due to association. Tribromobarbaloin itself is too sparingly soluble for molecular-weight determinations, but it is proposed to determine those of suitable derivatives.

If the views now stated are correct, the constitutions of barbaloin and of the other aloins must be more complex than has hitherto been assumed. The experimental investigation is being developed in various directions and it should be mentioned that Dr. A. Robertson is studying the synthesis of glucosides of rhein and of aloe-emodin.

EXPERIMENTAL.

The barbaloin used throughout this work was purified by two crystallisations from ethyl alcohol and dried over sulphuric acid. It was free from *iso*barbaloin.

Tribromobarbaloin, $C_{20}H_{15}O_9Br_3$.—To a solution of barbaloin (5 g.) in hydrobromic acid (*d* 1·5; 25 c.c.) cooled to 0°, bromine (8 g.) in hydrobromic acid (25 c.c.) was gradually added. No appreciable rise of temperature took place and after 4 hours the mixture was kept at room temperature for 4 days. The mixture was poured on ice, and the voluminous yellow precipitate (5 g.) separated and dried in air. When this was mixed with hot aqueous alcohol (60%, 50 c.c.), a clear reddish-brown solution was obtained, and this, after being boiled for a few minutes, deposited a fine yellow crystalline solid (the filtrate, *A*, was reserved). The bromo-compound was conveniently purified by extraction with acetone in a Soxhlet apparatus; it then separated from the solution as a bright yellow powder. When slowly heated, the compound decomposed with vigorous evolution of gas at 291° after darkening at 284°; on rapid heating, it decomposed without previous change at 296°. The compound is anhydrous and does not lose weight at 110° (Found: C, 37·7; H, 3·0; Br, 37·8, 37·6. Calc. for $C_{20}H_{15}O_9Br_3$: C, 37·5; H, 2·3; Br, 37·5%).

Tribromobarbaloin is extremely sparingly soluble in all the ordinary organic solvents and insoluble in water. It dissolves in much formic acid or acetic acid and separates slowly from the solutions; under these conditions, it appears to undergo some change, since the melting point falls to 265°. It is readily soluble in mineral

acids, the solution in concentrated sulphuric acid being reddish-yellow and slightly fluorescent. It is not attacked by the latter reagent (50%) when heated for a short time, nor does it undergo any change when heated at 110° with a methyl-alcoholic solution of hydrogen bromide (3%) in a sealed tube. It is readily soluble in alkalis, yielding golden-yellow solutions. Its alcoholic solution gives with ferric chloride a deep reddish-brown coloration; this becomes paler on warming and the original deeper colour reappears on cooling.

From the filtrate *A*, a small quantity of tribromonorbarbaloin was deposited on dilution with water.

Acetyltribromobarbaloin, $C_{20}H_9O_3Br_3(OAc)_6$.—The acetylation of tribromobarbaloin was investigated under various conditions, but a homogeneous product was obtained only by the following method. A solution of the bromo-compound (2 g.) in acetic anhydride (20 c.c.) to which two drops of sulphuric acid had been added was boiled for 2 hours. The cooled reaction mixture was poured into water; the heavy oil which was precipitated slowly solidified. The dried and finely powdered solid was purified by means of ligroin (b. p. 60—80°), from which it separated as an almost colourless powder; this was not definitely crystalline. It softened at 173° and melted at 185—187° to a yellow resin (Found: C, 43·4; H, 3·5; Br, 26·4. $C_{32}H_{27}O_{15}Br_3$ requires C, 43·1; H, 3·0; Br, 26·9%).

Acetyltribromobarbaloin is readily soluble in all the ordinary organic solvents with the exception of ligroin and *cyclo*hexane. It is readily attacked by chromic acid in acetic acid–acetic anhydride solution, yielding an amorphous powder which is insoluble in alkali and has not been purified.

Tribromobarbaloin Pentamethyl Ether.—Tribromobarbaloin in acetone was treated with potassium hydroxide solution (20%) and a large excess of methyl sulphate. The colourless oil that separated was converted into a flocculent solid when poured into water. The dried solid was triturated with ether, which left undissolved any partly methylated material; on evaporation, the filtered extract gave the methyl ether as a gum. In two experiments a solid was obtained which crystallised in well-defined prisms; it was very sparingly soluble in ether and apparently free from halogen, but it was not obtained in quantity sufficient for identification.

The *pentamethyl* ether separated as a colourless powder, m. p. 128—130°, when water was added to a solution in methyl alcohol–acetone [Found: C, 43·0; H, 4·0; Br, 33·3; OMe, 22·9. $C_{26}H_{27}O_9Br_3$ requires C, 43·1; H, 3·7; Br, 33·2; $(OMe)_5$, 21·4%]. It was somewhat sparingly soluble in methyl alcohol and insoluble in ligroin and water, but readily soluble in ether, ethyl alcohol,

acetone, and ethyl acetate. It was only very slowly attacked by potassium permanganate in hot acetone solution.

Acetyltribromobarbaloin Pentamethyl Ether.—When a solution of the preceding ether was treated with acetyl chloride, and the excess of acid chloride removed, the product obtained could not be freed from a red substance which was also formed. A pure product was, however, obtained by using acetic anhydride containing a few drops of pyridine. The *acetyl* derivative, a colourless powder, was purified by precipitation from its solution in methyl alcohol by water. It had m. p. 102—105° after softening at 90° and was readily soluble in all organic solvents except ligroin (Found : C, 44·1; H, 4·1; Br, 31·4; Ac, 6·4. $C_{28}H_{29}O_{10}Br_3$ requires C, 43·9; H, 3·8; Br, 31·4; Ac, 5·6%).

Tribromonorbarbaloin, $C_{16}H_{15}O_7Br_3$.—This was prepared by the bromination of barbaloin with bromine water in the manner described in the literature. After crystallising from alcohol and drying under reduced pressure, it had m. p. 193—194°, sintering at 188° and decomposing at 223° (Found : Br, 42·6. Calc. : Br, 42·9%). It can be recovered unchanged from its yellow solutions in alkalis on acidification.

Acetylation of Tribromonorbarbaloin.—Tribromotetra-acetylnorbarbaloin, $C_{16}H_{11}O_3Br_3(OAc)_4$, was obtained when tribromonorbarbaloin was acetylated with acetyl chloride. After crystallising from aqueous alcohol (70%), it had m. p. 137—139° (Found : Br, 32·5; Ac, 24·0. Calc. : Br, 33·0; 4Ac, 23·7%). It was insoluble in cold alkalis, but was hydrolysed to the parent bromo-compound by alcoholic sulphuric acid. It yielded an amorphous, dark brown powder when oxidised with chromic acid in acetic acid–acetic anhydride solution.

Tribromopenta - acetylnorbarbaloin, $C_{16}H_{10}O_2Br_3(OAc)_5$, was obtained when tribromonorbarbaloin was acetylated with acetic anhydride in the presence of sulphuric acid. It was sparingly soluble in cold aqueous alcohol (60%) and readily soluble in hot aqueous alcohol; from this solution it was deposited as an amorphous, granular, yellow powder, m. p. 139—140°, softening at 128° (Found : C, 40·5; H, 3·0; Br, 31·0. $C_{26}H_{25}O_{12}Br_3$ requires C, 40·6; H, 3·2; Br, 31·2%).

Tribromonorbarbaloin Pentamethyl Ether, $C_{16}H_{10}O_2Br_3(OMe)_5$.—Tribromonorbarbaloin was fully methylated only after two treatments with methyl sulphate and alkali in acetone solution. The *ether,* which separated as a colourless powder, m. p. 112—113° after softening at 101°, was readily soluble in organic solvents and was purified by the careful addition of water to its solution in acetic acid. The white powder, m. p. 115—116°, obtained became yellow

on exposure to air. It was stable to potassium permanganate in acetone solution. Two distinct preparations were analysed [Found: C, 39·5, 40·1; H, 3·6, 3·9; Br, 38·6, 37·8; OMe, 21·4. $C_{21}H_{25}O_7Br_3$ requires C, 40·0; H, 4·0; Br, 38·1; $(OMe)_5$, 24·6%].

The methyl ether cannot be reduced with zinc dust in the cold, but, on warming in acetic acid solution, bromine is eliminated, the product being an amorphous solid which has not been purified. The ether is insoluble in alkali, gives with nitric acid an intense purple-violet coloration and with sulphuric acid a deep purple, is somewhat readily attacked by potassium permanganate in acetone solution, and when oxidised with lead peroxide yields a substance which gives a green colour with sulphuric acid.

We desire to express our indebtedness to Messrs. Burroughs, Wellcome and Co. and to Dr. H. A. D. Jowett for generous gifts of material which have made this investigation possible. To Professor R. Robinson, F.R.S., we are also greatly indebted for the considerable help and suggestive criticism which he has freely given during the progress of the work. Part of the expense of the investigation has been met out of a grant from the Government Grant Committee of the Royal Society, which we gratefully acknowledge.

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