On comparing this behavior with that of β-chloro-acetyl fructose it does not appear anomalous and is indeed what would be expected of β-chloro-acetyl maltose.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE POLARIMETRY SECTION, BUREAU OF STANDARDS, UNITED STATES DEPARTMENT OF COMMERCE][1]

RELATIONS BETWEEN ROTATORY POWER AND STRUCTURE IN THE SUGAR GROUP. III.[2] THE BIOSE OF AMYGDALIN (GENTIOBIOSE) AND ITS CONFIGURATION

BY C. S. HUDSON

RECEIVED NOVEMBER 14, 1923

Haworth and Leitch[3] have recently applied Irvine's method of methylation and subsequent hydrolysis to the old problem of the determination of the structure of the biose of amygdalin, the glucoside of bitter almonds. It has long been known that the amygdalin molecule is composed of two molecules of *d*-glucose and one of *l*-mandelonitrile,

$$\underset{\text{(amygdalin)}}{C_{20}H_{27}O_{11}N} + 2\ H_2O = 2\ \underset{\text{(}d\text{-glucose)}}{C_6H_{12}O_6} + \underset{\text{(}l\text{-mandelonitrile)}}{C_6H_5.CHOH.CN}$$

By methylating amygdalin with dimethyl sulfate and sodium hydroxide solution it was transformed to the methyl ester of heptamethyl amygdalinic acid and this crystalline substance yielded on acid hydrolysis (1) *d,l*-mandelic acid, (2) 2,3,5,6-tetramethyl glucose and (3) a trimethyl glucose which was shown to have the methyl groups on Carbons 2, 3 and 5. The occurrence of racemic mandelic acid is explained by the racemizing and subsequent saponifying action of alkali on the *l*-mandelonitrile grouping in amygdalin. The tri- and tetramethyl glucoses that were found were the same substances that Haworth and Leitch[4] had previously isolated through the hydrolysis of fully methylated maltose, a fact which led them to express the conclusion:

"The disaccharide of amygdalin has therefore the structure of maltose and quite definitely cannot be cellobiose. For the stereochemical formulation of this maltose structure we are dependent on the researches of other workers on the selective action of enzymes, and here the results, if not conflicting, are certainly anomalous. Their results favor the view that the amygdalin biose is a glucose α-glucoside.....and therefore, on this reasoning, the biose itself must be maltose and amygdalin is mandelonitrile α-maltoside.....Should it ultimately be the case that the stereochemical representation of the biose is found to be that of a glucose β-glucoside, this cannot, of course, affect the structural formula we have herein ascribed to the sugar, but it may point to the identity of the amygdalin biose with isomaltose or gentiobiose."

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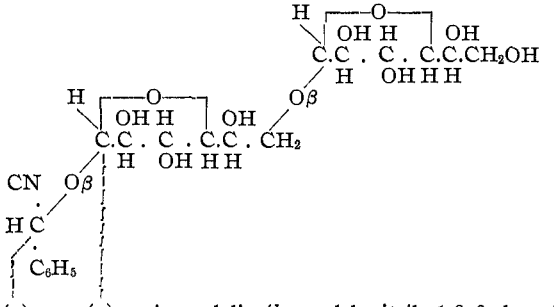
[1] Published by permission of the Director of the Bureau of Standards of the U. S. Department of Commerce.

[2] Part II immediately precedes this article.

[3] Haworth and Leitch, *J. Chem. Soc.*, **121**, 1921 (1922).

[4] Haworth and Leitch, *ibid.*, **115**, 809 (1919).

Supplementary evidence relating to the structure of the biose of amygdalin has been published quite recently by Kuhn,[5] who has applied E. F. Armstrong's method[6] of hydrolyzing a glucoside by an enzyme and observing the direction of the mutarotation of the liberated sugar. It will be recalled that Armstrong thus showed that the α and β forms of methyl glucoside liberate α- and β-glucose, respectively. Kuhn now shows that the two glucose molecules that are liberated during the hydrolysis of each molecule of amygdalin are both β-glucose and he concludes that the biose of amygdalin is a β-glucosido glucose. Adopting the maltose linkage (1 to 6) which has been proved by Haworth and Leitch for both maltose and the biose of amygdalin and considering both glucose residues to have the structure of β-glucose the configuration of amygdalin is to be written[7]



(y) (x) Amygdalin (*l*-mandelonitrile-1,6-β-glucosido-β-glucoside)

[5] Kuhn, *Ber.*, **56**, 857 (1923).

[6] Armstrong, *J. Chem. Soc.*, **83**, 1305 (1903).

[7] This configurational formula is the one which Kuhn has published with the exception that I have reversed the configuration of the *l*-mandelonitrile residue. The symbols (*x*) and (*y*) designate two carbon atoms which will later be subjects of discussion. The configuration here assigned to *l*-mandelonitrile is obtained from a consideration of the fact that the change from *l*-mandelic acid, which results from the saponification of *l*-mandelonitrile, to *l*-mandelamide is accompanied by a change of rotation in the dextro direction. This evidence, attention to which has been called by Freudenberg, Brauns and Siegel [*Ber.*, **56**, 193 (1923)], seems fully trustworthy. In a previous article [THIS JOURNAL, **40**, 813 (1918)] I have shown that the dextrorotatory amides of the acids of the sugar group have their α-OH on the right of the chain and the levorotatory ones have it on the left, and from this rule I deduced configurations for the two mandelic acids. My conclusions should, however, be reversed because I overlooked the significance of the fact that the mandelic acids are strongly rotatory in distinction from the weakly rotatory acids of the sugar group. Freudenberg has corrected my configurations and has shown that the rule which I proposed is to be considered a special case of the more general relation that *the change of the rotation of an α-hydroxy acid to that of its amide is in the dextro direction when the α-OH is on the right of the chain, the carboxyl group being written at the top, and in the levo direction when it is on the left of the chain.* This form of the rule is similar to that which I have proposed [THIS JOURNAL, **39**, 462, Footnote 3 (1917)] in the case of the acids and lactones of the sugar group.

The configuration that is assigned to the β-glucose in the amygdalin formula is that which has been proposed by Böeseken [*Ber.*, **46**, 2612 (1913)] and supported by Brigl [*Z. Physiol. Chem.*, **122**, 245 (1922)] from independent evidence.

Although the work of Haworth and Leitch, supplemented by the evidence that Kuhn has adduced, shows the configuration of amygdalin, the problem of the identity of the biose remains unsolved. Cellobiose has been excluded by Haworth and Leitch's results, as they have shown that it possesses a 1 to 5 linkage, while Kuhn's experiments have excluded maltose (an α-glucosido glucose), and while the biose may be isomaltose or gentiobiose, as stated by Haworth and Leitch, it may also be some undiscovered β-glucosido glucose. It is at this point that the following evidence from polarimetric data is presented, which shows clearly that the biose of amygdalin is gentiobiose. The proof of this identification is the fact, which will now be established, that *the rotation of the biose chain of amygdalin, as calculated from the rotations of two derivatives of amygdalin, namely, iso-amygdalin and prulaurasin, has the same value as the rotation of the chain of gentiobiose.* It will be seen from the calculations that this evidence is entirely independent of that of Haworth and Leitch and likewise of that of Kuhn, and that *the combining of the results of these three lines of evidence proves the configuration of gentiobiose to be that of 1,6-β-glucosido glucose.* Maltose and gentiobiose are thus shown to be the α- and β-glucosidic forms, respectively, of 1,6-glucosido glucose; they constitute the first α,β pair of compound sugars to be definitely allocated.

The Relationship of Amygdalin to Iso-Amygdalin and Prulaurasin

If the carbon atom marked (y) in the formula for amygdalin were symmetric it would be possible to apply to amygdalin the same method of calculation of its rotatory power that was used by Hudson and Johnson[8] to calculate the rotation of β-methyl gentiobiose. In that case the calculated $[\alpha]_D^{20}$ was —38°, in good agreement with the observed value, —36°. But the carbon (y) is asymmetric and its rotation is unknown. To get around the difficulty, advantage is taken of the fact that the *l*-mandelonitrile group in amygdalin is readily racemized by alkali, the remainder of the structure of the glucoside being unchanged, so that a new compound results, the iso-amygdalin of Dakin,[9] which is a mixture in nearly equal quantities of amygdalin (*l*-mandelonitrile bioside) and the corresponding *d*-mandelonitrile bioside. The last named substance has been crystallized from *iso*-amygdalin by Krieble[10] who speaks of it as *d*-amygdalin; this designation seems inappropriate and it is preferable to use the name neo-amygdalin previously proposed by Tutin,[11] who prepared its hepta-acetate in pure condition. A parallel series of three similar glucosides has been obtained in the same way by starting with *l*-mandelonitrile glucoside, a substance which Fischer prepared from amygdalin by the hydrolyz-

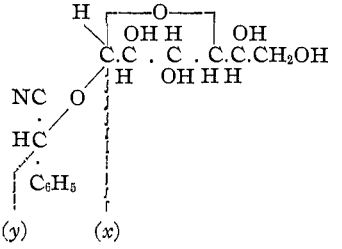
[8] Hudson and Johnson, THIS JOURNAL, **39**, 1272 (1917).

[9] Dakin, *J. Chem. Soc.*, **85**, 1512 (1904). See also Walker, *ibid.*, **83**, 472 (1903).

[10] Krieble, THIS JOURNAL, **34**, 716 (1912).

[11] Tutin, *J. Chem. Soc.*, **95**, 663 (1909).

ing action of yeast on the union between the two glucose molecules. Fischer's glucoside has the configuration



and is *l*-mandelonitrile β-glucoside. In alkaline solution its nitrile group racemizes[12] and a mixture of *l*- and *d*-mandelonitrile glucosides results. The mixture is the natural glucoside *prulaurasin* which Hérissey[13] isolated from the fresh leaves of *prunus laurocerasus*. Pure *d*-mandelonitrile glucoside is identical with the natural *sambunigrin* which Bourquelot and Danjou[14] isolated from the leaves of *sambucus nigra*.

The Rotatory Powers of the Glucosides of the Amygdalin Group

For the present calculations it is desirable to know with accuracy the rotations of iso-amygdalin (*d,l*-mandelonitrile β-bioside) and prulaurasin (*d,l*-mandelonitrile β-glucoside). It is assumed that the racemization of the nitrile group causes carbon (*y*) in their structures to be without rotation, an assumption which allows the calculations to be made. In Table I the values which have been accepted for the specific rotations of the six glucosides in question are recorded from the literature, and the data from which the values have been selected are stated in the footnotes.

TABLE I

THE ROTATIONS OF THE GLUCOSIDES OF THE AMYGDALIN GROUP

Substance	Mol. wt.	$[\alpha]_D^{20}$ in water	$[M]_D^{20}$ in water
Hexosides			
l-Mandelonitrile β-glucoside (Fischer's glucoside)....	295	−27[a]	− 8,000
d,l-Mandelonitrile β-glucoside (prulaurasin).....	295	−52[b]	−15,300
d-Mandelonitrile β-glucoside (sambunigrin).....	295	−76[c]	−22,400
Biosides			
l-Mandelonitrile β-gentiobioside (amygdalin).....	457	−38.5[d]	−17,600
d,l-Mandelonitrile β-gentiobioside (iso-amygdalin)...	457	−50.5[e]	−23,100
d-Mandelonitrile β-gentiobioside (neo-amygdalin)....	457	−61[f]	−27,900

[a] Fischer [*Ber.*, **28**, 1508 (1895)] found $[\alpha]_D^{20}$ = −26.9 and −26.8 for the glucoside which he made by the action of the enzymes of yeast on amygdalin. Fischer and Bergmann [*Ber.*, **50**, 1047 (1917)] found −27.0 for the substance which they prepared synthetically. The accepted value −27 seems certain within less than 0.5°.

[12] Caldwell and Courtauld, *J. Chem. Soc.*, **91**, 666, 671 (1907).

[13] Hérissey, *J. pharm. chim.*, [6] **23**, 5 (1906).

[14] Bourquelot and Danjou, *ibid.*, [6] **22**, 219, 385 (1906).

[b] Prulaurasin is doubtless a mechanical mixture. Hérissey found values of $[\alpha]_D$ ranging from —52.6 to —54.6, for the product which he isolated from *prunus laurocerasus*. Caldwell and Courtauld found —52.7 for the product made by the action of weak alkali on Fischer's glucoside, and Fischer and Bergmann found values ranging between —51.9 and —55.7 for the product which they synthesized. The average of the values for Fischer's glucoside (—27) and sambunigrin (—76, see below) is —51.5, which seems more trustworthy than the other values. The value —52 is accepted as probably correct within 1° for an equimolecular mixture of Fischer's glucoside and sambunigrin.

[c] Bourquelot and Danjou found —76.3 and —75.4 for the glucoside which they isolated from the leaves of *sambucus nigra*, and Fischer and Bergmann found —75.1 and —76.3 for the product which they synthesized. The accepted value —76 seems correct within 0.5°.

[d] While Fischer's glucoside, prulaurasin and sambunigrin are anhydrous substances, amygdalin crystallizes with three molecules of water. Caldwell and Courtauld's (Ref. 12, p. 673) value of —35.5 for this trihydrate (mol. wt., 511) corresponds to —39.7 for anhydrous amygdalin (mol. wt., 457). Auld [*J. Chem. Soc.*, **93**, 1277 (1908)] found —41.6, Schiff [*Ber.*, **32**, 2701 (1899)] —40.5, and Tutin —37.8 and —38.0 for the anhydrous substance. As Caldwell and Courtauld and Tutin clearly understood the nature of the partial racemization of amygdalin by alkali, a change which increases the levorotation, and apparently used much care in their measurements, their values are here accepted and the average —38.5 taken as probably correct within 0.5°, for anhydrous amygdalin.

[e] Dakin's value for iso-amygdalin dihydrate (mol. wt., 493) is —47.6, corresponding to —51.3 for the anhydride. The average of the accepted values for amygdalin and neo-amygdalin is —49.7, and the average of this and Dakin's value is —50.5, which seems probably correct within one degree.

[f] This is Krieble's value for anhydrous neo-amygdalin (Ref. 10, p. 727). The rotation varies considerably with the concentration and the temperature and this value is interpolated from Krieble's data. It is probably correct within 2°.

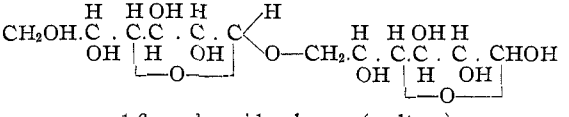
Calculation of the Rotation of the Biose of Amygdalin

Referring to the structural formula of amygdalin, let N represent the rotation of carbon (*y*), the asymmetric carbon of the *l*-mandelonitrile residue; let A represent that of carbon (*x*) and let X represent that of the remainder of the structure, the basal chain of the biose. The molecular rotation of amygdalin is then $(N + A + X)$. For neo-amygdalin the molecular rotation is $(-N + A + X)$, while for iso-amygdalin, which is assumed to be a mixture of equal parts of amygdalin and neo-amygdalin, the molecular rotation is $[(N + A + X) + (-N + A + X)] \div 2 = A + X = -23{,}100$, from Table I. Referring now to the structure of Fischer's glucoside, let N′ represent the rotation of carbon (*y*), A that of carbon (*x*), and B that of the remainder of the structure, the basal chain of glucose. The molecular rotation of Fischer's glucoside is then $(N' + A + B)$, that of sambunigrin is $(-N' + A + B)$ and that of their equimolecular mixture, prulaurasin, is $[(N' + A + B) + (-N' + A + B)] \div 2 = A + B = -15{,}300$, from Table I. Now the value of B, the basal chain of glucose, is known accurately from the molecular rotations of the α and β forms of

d-glucose to be +11,900;[15] hence A = —15,300—11,900 = —27,200. Substituting this value of A in the first equation of this section gives X = —23,100 + 27,200 = +4100 as the value of the molecular rotation of the basal chain of the amygdalin biose. As this sugar must unquestionably belong to the class of reducing sugars and be capable of existing in alpha and beta forms it is necessary, in order to be in a position to identify the sugar, to calculate the rotations of these forms by adding in one case and subtracting in the other the value of the rotation of the end asymmetric carbon atom of the reducing aldoses, which is known accurately for glucose as one-half the difference of the molecular rotations of its α and β forms, +8500. The molecular and specific rotations in water of the alpha and beta forms of the biose (mol. wt., 342) of amygdalin are thus calculated to be:

α Form of the biose, $[M]_D^{20}$ = +4100 + 8500 = +12,600, $[\alpha]_D^{20}$ = +37°
β Form of the biose, $[M]_D^{20}$ = +4100 − 8500 = —4400, $[\alpha]_D^{20}$ = —13°

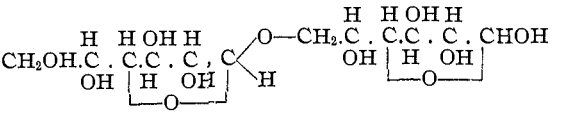
There are three known crystalline gluco-bioses of the reducing sugar type, namely, maltose, cellobiose and gentiobiose, and a fourth, isomaltose, which has not been obtained crystalline. The specific rotations of β-maltose and β-cellobiose are +118 and +16, respectively; hence neither of these sugars can be the biose of amygdalin. The stable rotation of isomaltose is about +60, which indicates that its β form must be much more dextrorotatory than is β-cellobiose, as the stable rotation of cellobiose is +35; this conclusion definitely excludes isomaltose as a possibility. On the other hand, crystalline β-gentiobiose rotates[16] approximately —11°, a value which agrees within the limits of error with the calculated value for the β form of the amygdalin biose. Amygdalin thus becomes *l-mandelonitrile-β-gentiobioside*, of the configuration which Haworth and Leitch and Kuhn have proved. In consequence, the structure of gentiobiose is now shown to be that of maltose, the two disaccharides possessing the same 1,6 linkage between their constituent glucose molecules. The difference between them consists in the stereochemical positions, maltose being *1,6-α-glucosido glucose* and gentiobiose being *1,6-β-glucosido glucose*. Their configurations are as follows, the formula for maltose being quoted from Haworth and Leitch.



1,6- α-glucosido glucose (maltose)

[15] Hudson, *J. Ind. Eng. Chem.*, **8**, 379 (1916). This is one-half the sum of the molecular rotations (mol. wt. 180) of the two forms, of $[\alpha]_D^{20}$ = 113 and 19, respectively.

[16] Bourquelot and Hérissey, *J. pharm. chim.*, [6] **16**, 418 (1902). Hudson, THIS JOURNAL, **38**, 1566 (1916). The stable rotation is +9.8°. The value —11 seems probably correct within 3°.



1,6- β-glucosido glucose (gentiobiose)

Maltose and gentiobiose thus constitute the first known α, β pair of compound sugars to be definitely allocated. Some deductions from this allocation will be discussed in a subsequent article.

Finally, mention may be made that the present proof of the identity of the biose of amygdalin with gentiobiose points the way to the synthesis of amygdalin and also to the preparation of gentiobiose from amygdalin. One should expect the synthesis to start with β-gentiobiose octa-acetate and proceed through the conversion of this to a halogen-acetyl gentiobiose,[17] which can doubtless be united with ethyl mandelate and the synthesis continued to amygdalin by the same reactions through which Fischer and Bergmann[18] have synthesized Fischer's glucoside, prulaurasin and sambunigrin from bromo-acetyl glucose and ethyl mandelate. To produce gentiobiose from amygdalin, one must seek to obtain an enzyme preparation, either from emulsin or from some other source, which hydrolyzes β-glucosides (or in particular Fischer's glucoside) but does not hydrolyze gentiobiose. There is little doubt that the hydrolyses are caused by separate enzymes, since Fischer has shown that amygdalin can be hydrolyzed to a mixture of Fischer's glucoside and glucose by the enzymes of yeast without the occurrence of any hydrolysis of the former substance.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF CORNELL UNIVERSITY]

ORTHO-CRESOL-TETRACHLOROPHTHALEIN AND SOME OF ITS DERIVATIVES

E. L. ARNOLD[1]

RECEIVED NOVEMBER 17, 1923

o-Cresol-tetrachlorophthalein was made for the purpose of comparing its absorption spectrum with that of phenolphthalein and phenol-tetrachlorophthalein. It was prepared by the method of Fraude[2] for making *o*-cresolphthalein using anhydrous stannic chloride as the condensing agent.

A mixture of 60 g. of *pure* *o*-cresol (2 molecular equivalents), 84 g. of *pure* crystallized

[17] Bromo-acetyl gentiobiose has been prepared in amorphous form by Hudson and Johnson, THIS JOURNAL, **39**, 1275 (1917).

[18] Table I, Footnote *a*.

[1] Work done in partial fulfilment of the requirement for the degree of Bachelor of Chemistry by E. L. Arnold, holder of the Grasselli Undergraduate Scholarship in Chemistry at Cornell University, 1922–1923.

[2] Fraude, *Am.*, **202**, 154 (1880).