CONFIGURATIONAL RELATIONSHIPS BETWEEN NATURALLY OCCURRING CYCLIC PLANT ACIDS AND GLUCOSE

TRANSFORMATION OF QUINIC ACID INTO SHIKIMIC ACID

by

GERDA DANGSCHAT AND HERMANN O. L. FISCHER

Department of Biochemistry, University of California Berkeley 4,
California (U.S.A.)

The old idea that meso-inositol could be formed by cyclization of D-glucose gained considerable strength by the determination of the configuration of meso-inositol[1] by GERDA DANGSCHAT, which later was confirmed by TH. POSTERNAK. Similar circumstances could be demonstrated in the field of cyclic plant acids, for instance Quinic Acid and Shikimic Acid. We were able to prove their constitution[2] and their planar configuration[3].

In 1937 we succeeded[4] by the degradation of shikimic acid into 2-desoxygluconic acid, IX[5] in demonstrating the same configuration for carbon atoms 3, 4 and 5 of shikimic acid as is found for carbon atoms 3, 4 and 5 of D-glucose.

An analogous relationship between quinic acid[6], which is more commonly found in the plant kingdom, and D-glucose, seemed very probable at that time. This physiologically important relationship could be established with certainty by transforming quinic acid into shikimic acid. In this communication we describe the successful transformation of derivatives of quinic acid into those of shikimic acid[7].

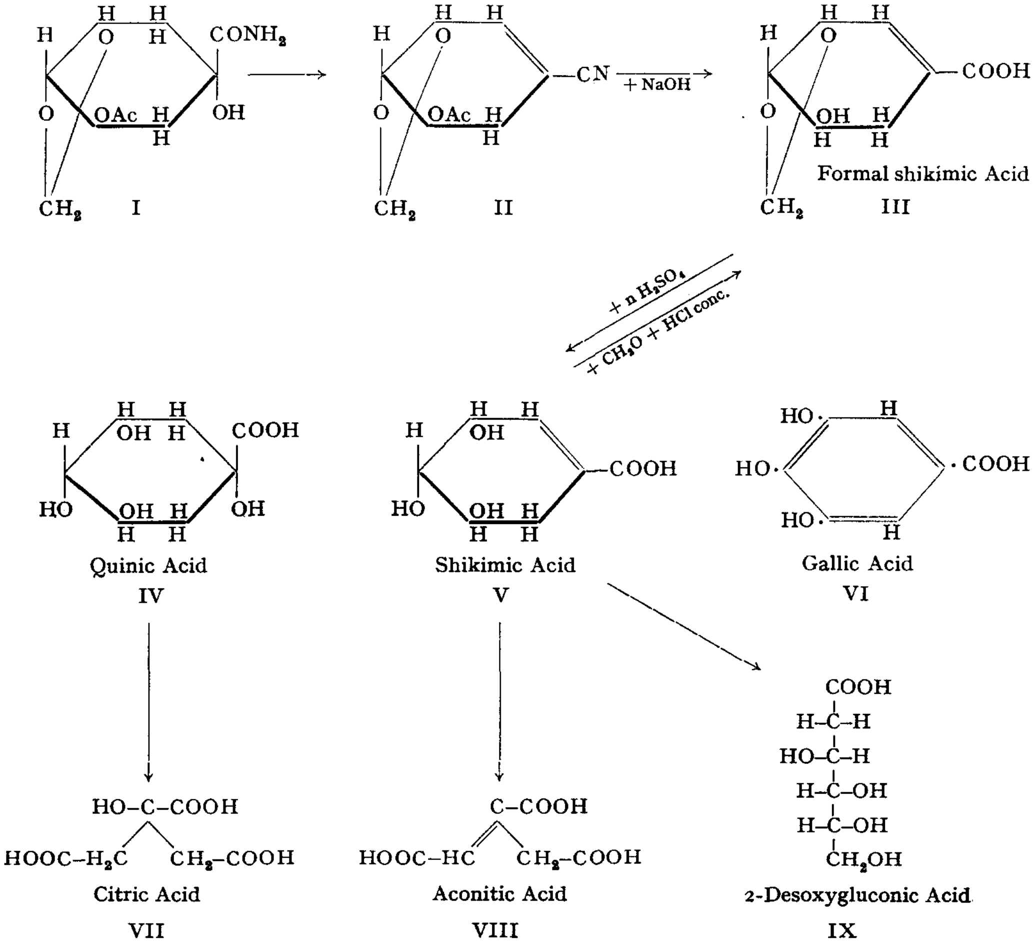
The use of the acetone compounds of quinic acid, which in previous work with these substances had proven highly satisfactory, met with unexpected difficulties. We therefore employed the formaldehyde derivatives which are described in the preceding paper[8], after having determined that the methylene group blocked the hydroxyls of carbon atoms 4 and 5 of the quinic acid as did the acetone.

We used α-toluene sulphonyl derivatives of quinic acid and found that the formation of a double bond by the splitting off of the toluene sulphonic acid by alkali only progressed smoothly after conversion to the nitrile, thus considerably weakening the stabilizing influence of the carboxyl group. By prolonged treatment of the 3-acetyl-4,5-formal quinic amide, I[9] with excess of *p*-toluene sulphonyl chloride and pyridine we performed three reactions in one operation: toluene sulphonylation of the amide, nitrilization of the amide, and finally the splitting off of the toluene sulphonyl group from the nitrile, with the result that the nitrile of the expected 3-acetyl-4,5-methylene *shikimic* acid, II, could be isolated. This could be converted by means of alkali into the methylene derivative of shikimic acid, III[10] which was transformed into free shikimic acid, V, in acid solution. The identification of shikimic acid was made by melting points, mixed melting points, and optical determinations.

References p. 203/204.

This shows conclusively that quinic acid bears the same steric relationship to D-glucose as that which has already been demonstrated for shikimic acid[11].

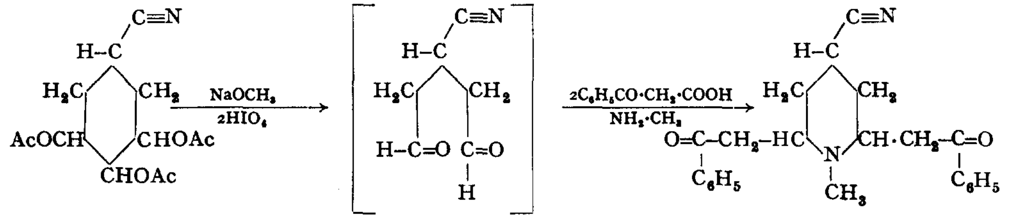
Furthermore, since the structural connection between quinic acid, IV, → shikimic acid, V, → gallic acid, VI, is obvious, it seems to us that in this chemical relationship we have an indication that many hydroaromatic and aromatic plant products are actually formed biologically from carbohydrates. In addition, it might be mentioned that our transformation in vitro of quinic acid to citric acid, VII, by means of periodic acid[12] has perhaps its biological counterpart in the work of Butkewitsch[13] who succeeded in establishing a connection between the fermentability of quinic acid and the formation of citric acid in the life of bacteria and fungi.



SUPPLEMENT

Our experiments described in this paper on the transformation of quinic acid into shikimic acid by splitting out water clearly show how strongly the carboxyl of the quinic acid influences its tertiary hydroxyl in the α position, and probably also the remainder of the molecule.

Our previous papers on the oxidation of quinic acid, shikimic acid and dihydro-shikimic acid by means of periodic acid have made available a series of 1.5-dialdehydes, which, depending on their origin, possess either a free or blocked hydroxyl and carboxyl group, or a carboxyl group alone. The possession of these aldehydes led us to an alkaloid synthesis, along the lines of the lobelanine synthesis of SCHÖPF[14]. The condensation, however, was successful only after the elimination of the electro-negative groups and the choice of a 1.5-dialdehyde which no longer contained any hydroxyl groups and only a carboxyl group in the form of its nitrile. This was the dialdehyde, XI, which is obtained by treating the nitrile of the dihydro-shikimic acid with 2 molecules of periodic acid[2,3,12].



X XI XII

Experimentally the synthesis was carried out in the following way: Triacetyl dihydro-shikimic acid amide, was transformed into the corresponding nitrile X, by heating with acetic anhydride. The nitrile was de-acetylated with a minimum amount of sodium methylate according to ZEMPLÉN, and the free nitrile was transformed into the dialdehyde, XI, by the action of 2 molecules of periodic acid. The dialdehyde was not isolated, but was condensed directly in aqueous solution with 2 molecules of benzoyl acetic acid ester and 1 molecule of monomethylamine at a p_H of 4.

The 3-cyano-lobelanine, XII, was isolated in a yield of 30% (calculated on the amount of triacetyl dihydro-shikimic acid nitrile), and showed the usual precipitation reaction of alkaloids, *e.g.* with perchloric acid, picric acid and picrolonic acid. It crystallized in long shining silklike needles similar to those of caffein, and showed a melting point of 143°.

EXPERIMENTAL

Preparation of the acetyl-methylene-shikimic acid nitrile from monacetyl-methylene-quinic acid amide

5 g monacetyl-methylene-quinic acid[15] were shaken with 10 g (2½ molecules) *p*-toluene sulphonyl chloride in 15 ml dry pyridine for a short time until dissolved. The brown coloured solution was kept for seven days at 37°. The solution was then diluted with 20 ml of water, and an oily substance separated. It was allowed to stand with occasional shaking for 15 minutes at room temperature in order to destroy any unused toluene sulphonyl chloride. The solution was then extracted twice with a large volume of chloroform. The united chloroform fractions were next shaken up with small portions of dilute sulphuric acid until all the pyridine was neutralized, and no more acid was used up. The solution was washed with a little water and then dried with sodium sulphate. The mixture was next filtered and the filtrate was evaporated in the vacuum of a water pump to remove all solvent. The light-brown oil (4.5 g) remaining was distilled under high vacuum. A light yellow oil (2.3–2.8 g, *i.e.*, 54–65% of the theoretical yield) distilled over at 0.2 mm and a bath temperature of 150–165°. It had a boiling point of 128°. After a second distillation it was almost colourless, but had a slight odour of toluene sulphonic acid and a minimum content of sulphur.

Preparation of unsaturated nitrile from monacetyl-isopropylidenequinic acid amide[16]

Reaction and processing follow exactly as described for the corresponding methylene compound. 4.3 g (*i.e.*, 77% of the theoretical yield) unsaturated acetyl-isopropylidene nitrile were obtained from 6.5 g monacetyl-isopropylidene-quinic acid amide. The compound had a light yellow colour and a boiling point of 125°/0.15 mm.

A sample twice redistilled was used for analysis:

5.068 mg gave 11.195 mg CO_2 and 2.860 mg H_2O;
3.169 mg gave 0.151 ml N_2 (26° and 741 mm).
$C_{12}H_{15}O_4N$ (237.1): Calc. C 60.7 H 6.4 N 5.9
Found C 60.8 H 6.3 N 5.4

Hydrolysis of acetyl-methylene-shikimic acid nitrile to methylene-shikimic acid

3.3 g distilled acetyl-methylene-shikimic acid nitrile were boiled for two and a half hours with 45 ml N sodium hydroxide (about 3 molecules). A condenser was attached to take off the water vapours and the ammonia. Water was added to the distillation flask during boiling so that the volume was not reduced below one-half the original. The condensate was caught in an ice-cooled receiver and at the end of the time the ammonia could be determined almost quantitatively. No formaldehyde was found in the distillate even after acid hydrolysis. The reaction liquid, which was coloured dark brown, was cooled and the alkali was neutralized by addition of 41 ml N sulphuric acid and 4 ml N hydrochloric acid. The weak acetic acid solution was reduced to dryness in the best vacuum obtainable by a water pump, during which time the bath temperature was not allowed to rise above 35°. The residue was extracted thoroughly several times with ethyl acetate, and the united filtered extractions were evaporated under reduced pressure. If crystals are at hand for inoculation, the yellow syrup remaining will begin to crystallize on inoculating. 33–38% of the theoretical yield of crystallized methylene-shikimic acid was obtained from the concentrated ethyl acetate solution, but these crystals still had a yellow colour. Using animal charcoal, a recrystallization from ethyl acetate was made for further purification.

The substance, well crystallized in rhombic plates, had a m.p. of 138° and showed no depression of the melting point on addition of an equal quantity of a preparation made from shikimic acid. The preparation twice recrystallized gave in aqueous solution the following rotation:

$$[\alpha]_D^{19°} = -88.7°$$[17] $$(1 \text{ dm tube}, c = 2.17, \alpha_D^{19} = -1.93°).$$

Further quantities of the acid could be obtained from the motherliquor of the isolated methylene-shikimic acid in the following manner: The methylene-shikimic acid methyl ester was formed by esterification with diazomethane and was distilled under a high vacuum at a bath temperature of 170–190°. It was then kept for two to three days at 37° together with pyridine and toluene sulphonyl chloride. The toluene sulphonyl-methylene-shikimic acid ester (m.p. and m.p. of the mixture 118–119°) crystallized out readily on gradual addition of water and trituration. This isolated quantity corresponds to a further 15–20% of the theoretical yield of methylene-shikimic acid, so that together about 52% of the acid obtained from the nitrile can be definitely identified as a derivative of the shikimic acid. The methylene-shikimic acid is easily isolated and identified by preparing its toluene sulphonyl-methyl ester, which readily crystallizes. This process is to be recommended, if no inoculation crystals of the free methylene-shikimic acid are at hand or if difficulties appear during the isolation of the free acid. After washing with 50% alcohol the ester is at once obtained in the pure state. For analysis and optical determinations it has to be recrystallized once more from alcohol:

5071 mg substance gave 10.090 mg CO_2 and 2.350 mg H_2O
7921 mg substance gave 5170 mg $BaSO_4$
$C_{16}H_{18}O_7S$ (354.2): Calc. C 54.2 H 5.1 S 9.1
Found C 54.2 H 5.2 S 9.0
$[\alpha]_D^{24°} = -42.5°$ (in chloroform)[18] (1 dm tube, c = 3.25, $\alpha_D^{24} = -1.38°$).

Hydrolysis of the unsaturated acetyl-isopropylidene nitrile

The hydrolysis of the unsaturated isopropylidene nitrile can be carried out under the same mild conditions as the corresponding methylene compound. In this reaction the ammonia can also be determined nearly quantitatively after about two hours boiling with dilute alkali. Furthermore, it was found that 25% of the theoretically possible amount of acetone was split off by the alkali. The acetone could be determined in the distillate by titration with alkaline hypoiodite solution and identified as the *p*-nitro- or dinitrophenylhydrazone. The further processing parallels the procedure used for the methylene nitrile. From the acetonated compound, however, it was not possible to isolate the free acetonated acid, nor to crystallize a derivative of the acetonated unsaturated ester, which had been obtained by esterification with diazomethane and subsequent distillation in a high vacuum. If, however, the unsaturated ester, of which 27% of the theoretical yield was obtained by distillation, is hydrolysed by acetic acid, about 4.5% of the theoretical amount (based on the amount of nitrile used) is obtained in crystallized form[19] as shikimic acid methyl ester. After two recrystallizations from ethyl acetate and ligroin, the m.p. was 112–114° and there was no depression of the melting point when the substance was mixed with equal amounts of a compound prepared from shikimic acid for comparison.

SUMMARY

The transformation of quinic acid into shikimic acid by means of the methylene derivatives of these acids has been described. Thus the configuration of the carbon atoms 3,4 and 5 of quinic acid has been shown to be the same as in shikimic acid, which had previously been configurationally related to D-glucose.

3-cyano-lobelanine has been synthesized from dihydroshikimic acid nitrile, benzoyl acetic acid, and monomethyl amine under conditions sufficiently mild so that they might exist in plant or animal organisms.

RÉSUMÉ

Nous avons décrit la transformation de l'acide quinique en acide shikimique à l'aide des dérivés méthyléniques de ces acides. Nous avons montré ainsi que la configuration des atomes de carbone 3, 4 et 5 dans l'acide quinique est la même que dans l'acide shikimique, dont la configuration avait été précédemment reliée à celle du D-glucose.

La 3-cyano-lobélanine a été synthétisée à partir du nitrile de l'acide dihydro-shikimique, de l'acide benzoylacétique et de la monométhylamine sous des conditions suffisamment douces pour exister dans l'organisme végétal ou animal.

ZUSAMMENFASSUNG

Wir beschreiben die Umwandlung der Chinasäure in die Shikimasäure über die entsprechenden Methylenderivate. Es wurde also gezeigt, dass die Konfiguration der Kohlenstoffatome 3, 4 und 5 in der Chinasäure dieselbe ist wie in der Shikimasäure, deren Konfiguration schon früher auf die der D-Glucose zurückgeführt wurde.

3-Cyanolobelanin wurde aus Dihydroshikimisäure-nitril, Benzoylessigsäure und Monomethylamin unter milden Bedingungen synthetisiert, wie sie auch im pflanzlichen oder tierischen Organismus vorkommen können.

REFERENCES

[1] G. Dangschat, *Naturwisenschaften* ,30 (1942) 146; *C. A.*, 37 (1943) 3408^6.
Th. Posternak, *Helv. Chim. Acta*, 25 (1942) 746.
Confer also H. O. L. Fischer, *Harvey Lectures*, Ser. 40 (1945) 156–178.
H. G. Fletcher Jr, *Advances in Carbohydrate Chem.*, Vol. 3, Academic Press, Inc., New York 1948.

[2] H. O. L. Fischer and G. Dangschat, *Ber.*, 65 (1932) 1009 and *Helv. Chim. Acta*, 17 (1934) 1200.

[3] H. O. L. Fischer and G. Dangschat, *Helv. Chim. Acta*, 18 (1935) 1206.

[4] H. O. L. Fischer and G. Dangschat, *Helv. Chim. Acta*, 20 (1937) 705.

[5] Max Bergmann *et al.*, *Ber.*, 55 (1922) 158; *Ber.*, 56 (1922) 1052.
P. A. Levene and G. Mikeska, *J. Biol. Chem.*, 88 (1930) 791.

[6] Quinic acid occurs not only in the free state in the plant kingdom but also for example in chlorogenic acid as a depside with caffeic acid. For the constitution of chlorogenic acid cf. H. O. L. Fischer and G. Dangschat, *Ber.*, 65 (1932) 1037.

[7] A preliminary notice on the same subject has been published in *Die Naturwissenschaften*, 26 (1938) 562.

[8] *9th Communication on Quinic Acid and derivatives*, *J.A.C.S.*, in press.

[9] *9th Communication on Quinic Acid and derivatives*, *J.A.C.S.*, in press.

[10] *9th Communication on Quinic Acid and derivatives*, *J.A.C.S.*, in press.

[11] This relationship is also a confirmation of the assumption of the cis position of the hydroxyls 4 and 5 of quinic acid and shikimic acid which we have always made on the basic of the work of Boeseken (cf. also Hückel, *Theoretische Grundlagen der Chemie*, 1 (65–66).

[12] H. O. L. Fischer and G. Dangschat, *Helv. Chim. Acta*, 17 (1934) 1196. Cf. also shikimic acid → aconitic acid, VIII, H. O. L. Fischer and G. Dangschat, *Helv. Chim. Acta*, 18 (1935) 1204.

[13] Wl. Butkewitsch, *Biochem. Z.*, 145 (1924) 442.

[14] C. Schöpf and G. Lehmann, *Liebig's Ann.*, 518 (1935) 1–37.

[15] See *9th Communication on Quinic Acid and derivatives*, *J.A.C.S.*, in press.

[16] H. O. L. Fischer and G. Dangschat, *Ber.*, 65 (1932) 1020. The yield is increased if the processing is performed two hours after action of the acetylation reagent.

[17] See *9th Communication on Quinic Acid and derivatives*, *J.A.C.S.*, in press.

[18] After acid hydrolysis following the prescription given in the *"9th Communication on Quinic Acid and derivatives"* free shikimic acid is obtained:

its m.p. and m.p. of a 50% mixture 184–185°; $[\alpha]_D^{19°} = -183°$
(in water, 1 dm tube, c = 1.23, $\alpha_D^{19} = -2.25°$).

[19] The small yield of *crystallized* substance suggests that the acetyl-isopropylidene-shikimic acid nitrile contains, unlike the corresponding methylene compound, a considerable quantity of a 1,2 unsaturated product.

Received June 23rd, 1949