673. *Quassin and Neoquassin. Part I.*

By E. London, Alexander Robertson, and (in part) H. Worthington.

Quassin $C_{20}H_{22}O_4(OMe)_2$ and neoquassin $C_{20}H_{24}O_4(OMe)_2$ have been isolated and separated by two procedures. The former compound is an unsaturated lactone containing two (and possibly three) *C*-methyl groups, a tertiary hydroxyl, and a ketonic group. On hydrogenation it gives a dihydro-derivative and on reduction forms neoquassin. With hot mineral acids quassin is demethylated, giving nor- and bisnor-quassin whilst with warm alcoholic potassium hydroxide it gives an unsaturated acid *iso*quassinic acid.

Neoquassin $C_{20}H_{24}O_4(OMe)_2$, which forms quassin on oxidation, is an unsaturated ketone containing two hydroxyl groups of which one reacts to form a monomethyl and a monoethyl ether. Partial demethylation of neoquassin gives norneoquassin whilst dehydrogenation yields a mixture from which the monomethyl ether of an alkyl-dihydroxybenzene has been isolated.

The resolution of the mixture of bitter constituents of Quassia wood into pure chemical compounds has hitherto proved a major obstacle to the elucidation of the chemistry and constitution

of these substances. An examination of the earlier work by Winckler (*Rep. Pharm.*, 1835, [2], **54**, 85; 1839, [2], **65**, 74), Wiggers (*Ann. Pharm.*, 1837, **21**, 40), Christensen (*Arch. Pharm.*, 1882, [3], **220**, 481), Oliveri and Denaro (*Gazzetta*, 1884, **14**, 1; 1885, 15, 6), Oliveri (*ibid.*, 1887, **17**, 540; 1888, **18**, 169), Adrian and Moreaux (*Pharm. J.*, 1884, **3**, **14**, 507), and Massute (*Arch. Pharm.*, 1890, **228**, 147) on the isolation and characterisation of the constituents of Quassia wood shows that there is a considerable lack of uniformity regarding the nature of their products. In a more recent examination of the crystalline bitter constituents of *Quassia amara*, Clark (*J. Amer. Chem. Soc.*, 1937, **59**, 927, 2511) obtained quassin, $[\alpha]_D^{20}$ +39·8°, m. p. 205—206°, and neoquassin, $[\alpha]_D^{20}$ +46·6°, m. p. 225—226°, which he considered to be isomeric and to have the formula $C_{20}H_{24}O_4(OMe)_2$. From the wood of *Picraena excelsa*, a tree closely related to *Quassia amara*, this author obtained a third isomeride, $[\alpha]_D^{20}$ +45·4°, m. p. 218°, stated to be the main constituent, which he designated picrasmin, a name originally applied by Massute (*loc. cit.*) to two closely related products, m. p. 204° and m. p. 209—212°, from the same source.

In our first approach to the problem of isolating and purifying the constituents of Quassia wood, *Quassia amara* L., we employed methods similar to those of Clark (*loc. cit.*) but with a somewhat simpler procedure for the fractional crystallisation by which two products were obtained [(*A*), m. p. 213·5°, and (*B*), m. p. 219°] from the crude extract. The same products were obtained from samples of Merck's (Germany) crystalline and amorphous commercial quassin and, in contrast to Clark's findings (*loc. cit.*), from *Picraena excelsa* Planch. Subsequently, in the course of many attempts to find a more rapid procedure for the isolation and purification of the constituents of Quassia wood, it was discovered that a crude crystalline, partially purified, extract could be separated by chromatography from chloroform on alumina into two bitter constituents. Of these, the somewhat less strongly adsorbed compound, for which we have retained the name quassin, m. p. 222°, $C_{20}H_{22}O_4(OMe)_2$, would appear to be identical with *iso*quassin which Clark (*loc. cit.*) prepared by the action of chromic acid on both his quassin, m. p. 205—206°, and his neoquassin, m. p. 225—226°, and to which he ascribed the empirical formula $C_{20}H_{24}O_4(OMe)_2$. The second constituent, $C_{20}H_{24}O_4(OMe)_2$, m. p. 228°, is very similar to, if not identical with, Clark's neoquassin and we have retained this author's designation. In addition there was isolated a minute amount of a brownish-yellow pigment of which the main component is a bright-yellow substance, m. p. 254°; an examination of this pigment is reserved for a later communication. By the chromatographic procedure the products (*A*) and (*B*) were found to be mixtures—(*A*), m. p. 213·5°, contained quassin with 35—36% of neoquassin, and (*B*) mainly neoquassin with a little quassin, approximations in agreement with the composition of (*A*)and (*B*) estimated from the mixed melting-point curve of pure quassin and neoquassin.

In the separation of quassin and neoquassin by chromatography it was not found possible to differentiate between the zones derived from an initially colourless mixture. The division of the columns had, therefore, to be made arbitrarily and it was only after much experimental work under standard conditions that pure compounds could be isolated by this tedious method. Other routes were, therefore, explored and ultimately, when it was discovered that, unlike neoquassin, quassin was a lactone, a highly satisfactory and comparatively rapid method of separating the components of a crude or partly purified crystalline extract of the wood was devised. Although insoluble in cold dilute aqueous potassium hydroxide, quassin dissolves readily in cold 5% methanolic potassium hydroxide and is not precipitated on dilution with water or extracted from the resulting aqueous methanolic solution with solvents. Thus, when a solution of the quassin–neoquassin mixture in 5% methanolic potassium hydroxide (equivalent amount) is diluted with water the neoquassin only is precipitated After the separation of the neoquassin the quassin is recovered from the alkaline liquor by precipitation with carbon dioxide. A detailed examination of the products obtained by this route with those isolated by chromatography failed to show any divergence in their properties.

Neoquassin occasionally crystallised in a metastable form (plates, m. p. 213°) which did not always readily pass into the stable form (thick prisms, m. p. 228°) when recrystallised. Clearly this property was one of the factors complicating the resolution of quassin–neoquassin mixtures by fractional crystallisation.

In addition to the main constituents the alkali method led to the isolation of a small amount of a new alkali-soluble compound, m. p. 246—248° (decomp.), which appears to have the empirical formula $C_{19}H_{20}O_4(OMe)_2$.

Quassin, m. p. 222°, is a dextrorotatory unsaturated lactone which contains two methoxyl groups, one double bond as estimated by the perbenzoic acid method, one active hydrogen

atom as determined by the Zerewitinoff method, and at least two, and possibly three, *C*-methyl groups according to the Kuhn–Roth method As it does not give formaldehyde or acetone on ozonolysis quassin is devoid of a vinyl or *iso*propylidene group. With Tollens's reagent and with the usual tests for sterols including the Liebermann–Burchardt reaction quassin gives negative results but in the Legal test for unsaturated lactones (cf. Jacobs *et al.*, *J. Biol. Chem.*, 1926, **70**, 1) it gives a reddish coloration in pyridine similar to that given by coumarin and angelicalactone. With alcoholic 2 : 4-dinitrophenylhydrazine hydrochloride quassin gives an amorphous orange product which after purification by chromatography did not crystallise. On the other hand, a crystalline 2 : 4-dinitrophenylhydrazone of slightly lower melting point was separated from the reaction mixture and, although this could not be recrystallised, it seems clear that quassin contains a ketonic group, a view supported by the behaviour of certain quassin derivatives in readily forming amorphous 2 : 4-dinitrophenylhydrazones. Thus, of the six oxygen atoms, two are present in methoxyl groups, two in the lactone system, one as a keto-group (quassin does not give reactions of an aldehyde), and one as a hydroxyl group which is apparently tertiary since quassin cannot be acylated under the usual conditions.

Characteristic of quassin is the ease with which it is demethylated with warm acidic reagents. When the compound is heated with 3·5% or 4% hydrochloric acid the main product of the reaction is norquassin $C_{20}H_{23}O_5(OMe)$ which behaves like a monohydric phenol, giving a pale red-violet ferric reaction and forming an acetate, and an orange-red product with benzenediazonium chloride. On treatment with an excess of diazomethane norquassin is partly converted into quassin but the main product of the reaction is an alkali-soluble, phenol-like isomeride of quassin which, like norquassin, is precipitated from its alkaline solution with carbon dioxide. This product, however, does not react with benzenediazonium chloride or form an acetate under the usual conditions. With a warm mixture of concentrated hydrochloric and acetic acid quassin is demethylated, giving bisnorquassin which gives a very weak ferric reaction, rapidly reduces Fehling's solution, and forms a dioxime. Bisnorquassin, which on titration behaves as a monobasic acid, readily forms a diacetate, but with an excess of ethereal diazomethane appears to give only a monomethyl ether $C_{21}H_{23}O_5(OMe)$ which is not identical with the isomeride formed by the methylation of norquassin. Bisnorquassin, which may have been the main constituent in quassic acid, m. p. 245° (Oliveri and Denaro, *loc. cit.*, and Oliveri, *loc. cit.*), is probably identical with Clark's " quassinol," m. p. 263° (*loc. cit.*), obtained from impure quassin, m. p. 205—206°, but the name applied by this author is not strictly definitive.

Although with aqueous-alcoholic sodium or potassium hydroxide quassin readily gives solutions of the corresponding salts of quassinic acid, the liberation of the parent acid results in the immediate regeneration of quassin. It was found, however, that on being boiled with 5% alcoholic potassium hydroxide quassin gave rise to a stable unsaturated monobasic acid, *iso*quassinic acid, $C_{20}H_{24}O_5(OMe)_2$, together with a small amount of a second monobasic acidic product which has not yet been defined. *iso*Quassinic acid, which is conveniently characterised by the formation of the methyl ester, readily forms an amorphous 2 : 4-dinitrophenylhydrazone and therefore appears to have retained the keto-group of quassin. As methyl *iso*quassinate is insoluble in alkalis the hydroxyl group liberated by the opening of the lactone ring is alcoholic in character. On being heated *iso*quassinic acid loses water, giving a neutral lactone which is not identical with quassin. When an alkaline alcoholic solution of crude potassium *iso*quassinate was diluted with water and kept at room temperature for a long period oxidation occurred, giving a complex acidic mixture of which the main constituent was a dibasic acid, $C_{18}H_{21}O_4(OMe)(CO_2H)_2$ or $C_{18}H_{23}O_4(OMe)(CO_2H)_2$, characterised by the formation of a dimethyl ester.

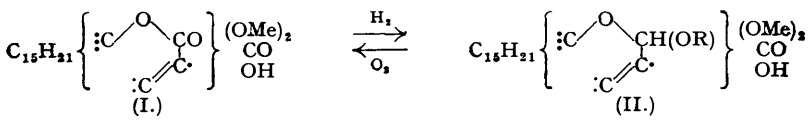
Although attempts to hydrogenate quassin with a palladium–charcoal catalyst in neutral solution were unsuccessful it was found that in alkaline solution containing this catalyst it readily absorbed hydrogen giving rise to dihydroquassin, a neutral saturated compound which formed an amorphous 2 : 4-dinitrophenylhydrazone. Similarly, with aqueous sodium *iso*-quassinate the product was a saturated ketonic acid which has not yet been obtained pure. In numerous experiments on the reduction of quassin sodium amalgam was found to give an amorphous product but when a highly active Raney nickel catalyst and hydrogen at atmospheric pressure were employed some neoquassin was formed. In attempts to improve the yield of the latter compound the use of hydrogen at 60 lb. per sq. in. gave an oily product containing a solid, m. p. 210—211° (decomp.).

Neoquassin, m. p. 228°, is an unsaturated neutral compound containing one double bond, two hydroxyl groups (by the active hydrogen method), two, and possibly three, *C*-methyl

groups, and a ketonic group which reacts to form an amorphous 2 : 4-dinitrophenylhydrazone. Neoquassin, which is devoid of a vinyl or *iso*propylidene group, gives negative results with sterol reagents except the Liebermann–Burchardt test, in which, unlike quassin, it gives a magenta colour. It is also distinguished from quassin in giving a positive Tollens and a negative Legal reaction in pyridine as well as by the fact that it is not affected by boiling 5% alcoholic-potassium hydroxide. On treatment with hot 3·5% or 15% hydrochloric acid neoquassin is partly demethylated, giving alkali-soluble norneoquassin $C_{20}H_{25}O_5(OMe)$, but bisnorneoquassin has not yet been obtained. Norneoquassin, which gives a deep violet-red ferric reaction, reacts with benzenediazonium chloride (azo-dye formation) and forms an acetate which is amorphous but which on deacetylation regenerates the parent compound. Of the two hydroxyl groups in neoquassin one is reactive since the compound forms an ethyl ether with warm alcoholic hydrochloric acid and an acetate. As quassin does not behave in this manner it is clear that the hydroxyl group involved in these reactions is generated in the reduction of quassin to neoquassin. This view is confirmed by the observation that on oxidation with chromic acid neoquassin is converted into quassin, identical with the natural compound. In the ethylation of neoquassin a small amount of a second product has been isolated which has not yet been examined in detail but is considered to be an isomeride of the ethyl ether. When methanolic hydrochloric acid is employed *O*-methylneoquassin is formed but this has not yet been resolved into two components.

In exploratory experiments on the dehydrogenation of quassin and neoquassin it has been found that when heated with palladium–charcoal neoquassin gives two neutral products which, from their composition, appear to contain the major part of the neoquassin molecule with but little loss of hydrogen. With selenium a phenolic fraction was formed from which a small amount of a monohydric phenol $C_9H_{11}O(OMe)$, m. p. 70°, was isolated. From a comparison of its ultra-violet absorption spectrum with the spectra of quinol, catechol, resorcinol, guaiacol, and dihydroeugenol, this phenol is regarded as being an alkyl derivative of either guaiacol (supported by ferric reaction) or resorcinol monomethyl ether rather than of quinol monomethyl ether. This product does not appear to be identical with either of the known isomeric alkyl derivatives of guaiacol or resorcinol monomethyl ether, and a direct comparison has shown that it is not identical with synthetical 2-hydroxy-3-methoxy- or 3-hydroxy-2-methoxy-*n*-propylbenzene (private communication from Dr. J. P. Brown, late of this laboratory) or with 4-hydroxy-3-methoxy*iso*propylbenzene, the synthesis of which will be described in a subsequent communication.

From the properties of quassinic acid, more especially its behaviour with alkalis, it seems probable that quassin is a lactone of the γ- or δ-type. The regeneration of quassin from the salts of this acid with carbon dioxide find a close analogy in the behaviour of sodium *o*-hydroxycinnamates in giving the corresponding coumarins under similar conditions. Consequently the double bond in quassin may be in the $\alpha\beta$-position to the carbonyl group of the lactone system, a view which is in agreement with the positive Legal test (Jacobs *et al.*, *loc. cit.*) and with the resistance of the compound to hydrogenation in neutral media in conjunction with the ready hydrogenation of sodium quassinate. On this basis, and on the assumption that the action of warm alcoholic potassium hydroxide does not modify the fundamental structure of quassinic acid, *iso*quassinic acid can be regarded as the *trans*-form of the parent acid.



The etherification of neoquassin with alcoholic hydrochloric acid is reminiscent of the formation of acetals and thus we may consider *O*-ethylneoquassin to be a mixed acetal analogous to the methylglucosides and to be represented by a formula of the type (II; R = Et) with neoquassin as type (II; R = H), structures which are in keeping with the resistance of neoquassin and its ethers to the action of warm alcoholic potassium hydroxide. Further, this type of structure (II; R = H) for neoquassin also affords a satisfactory explanation of its oxidation to quassin of type (I) (cf. oxidation of *O*-dimethylcitromycinol, *J.*, 1950, 1031).

Moreover, although the behaviour of quassin with alcoholic alkalis is consistent with the compound's being an acidic enol, the latter group is excluded by the failure of quassin to give a ferric reaction and by the fact that a secondary alcohol arising by its reduction would not be expected to etherify under the conditions employed in the formation of *O*-ethylneoquassin. Chromophorically the difference arising from the change ·CO·O· ⟶ ·CH(OH)·O· would be expected to be slight, and in agreement with the relationship between the two compounds now postulated the ultra-violet absorption spectra of quassin and neoquassin are almost identical. On the basis of the lactone–hemiacetal relationship suggested for quassin and neoquassin, the conversion of the system ·CO·O· into ·CH(OH)·O· involves the introduction of a new asymmetric carbon atom and hence neoquassin might be expected to exist in two forms capable of separation. Although this has not yet been detected the formation of two products on etherification of neoquassin may be found ultimately to arise in this way.

With regard to the methoxyl groups in quassin and neoquassin, which in their behaviour with acidic agents resemble a carbomethoxy-group, it seems justifiable to assume that they are present in a benzenoid or potential benzenoid system appearing as the phenol, m. p. 70°, obtained by dehydrogenation. In support of the compounds' containing a dimethoxybenzenoid system it may be noted that the demethylation of certain veratrole derivatives and related ethers under comparatively mild conditions (Robinson and Cardwell, *J.*, 1915, **107**, 257; cf. Kostanecki and Edelstein, *Ber.*, 1905, **38**, 1507, and Stoermer, *Ber.*, 1908, **41**, 323) is somewhat analogous to the behaviour of quassin and neoquassin with warm acids. An alternative suggestion is that quassin and neoquassin contain a dihydrobenzenoid system and hence are di-enolic ethers of the type (III) or (IV) where the combined arrangement of the constituents R, R^1, R^2, and R^3 is such that the system cannot readily aromatise under the influence of acids. This type of structure would account for the behaviour of the compounds with acids and, in particular, for the properties of the highly acidic bisnorquassin which to some extent resembles dihydroresorcinol. Unless a deep-seated change in the quassin molecule is caused by warm acids it is clear that the introduction of the second active carbonyl group, concerned in oxime formation by bisnorquassin, is a direct result of demethylation. An objection to the dihydrobenzenoid hypothesis is the fact that enolic ethers of the type (III) or (IV) are generally much more sensitive than quassin or neoquassin to fission with warm alkaline reagents. With regard to the carbon skeleton present in quassin and neoquassin it is clear that, if the compounds contain an aromatic-ring system, either attached at one carbon atom of or fused to another cyclic system, then, from the empirical formulæ and the functional groups present, the molecular structures can only contain one additional carbocyclic system together with the lactone ring. If, on the other hand, the compounds are dihydrobenzenes, type (III) or (IV), there must be two additional carbocyclic rings and the lactone system present.

The results described in the present work differ considerably from those obtained in the extensive experimental work of Clark (*loc. cit.*) Of the isomeric products isolated by this author it is now clear that the so-called quassin, m. p. 205—206°, and picrasmin were mixtures of quassin, m. p. 222°, and neoquassin, m. p. 228°, and that the composition of the mixture, m. p. 205—206°, can be changed by fractional crystallisation. Further, the wood of *Picraena excelsa* Planch. contains both quassin and neoquassin. Of the other hand, Clark's neoquassin appears to have been almost pure. As can be explained on our results Clark, by means of chromic acid, was able to convert his quassin and neoquassin into *iso*quassin, m. p. 221°, which is almost certainly identical with quassin, m. p. 222°, but for which Clark adopted the empirical formula $C_{22}H_{30}O_6$ and not $C_{22}H_{28}O_6$, remarking on the curious nature of the reaction. The identity of Clark's *iso*quassin with pure quassin appears to be supported by his preparation of " quassinol " $C_{20}H_{24}O_6$, m. p. 263°, for which we prefer the more definitive term bisnorquassin. Semidemethoxyquassin $C_{21}H_{28}O_6$, m. p. 213°, which Clark obtained from his quassin, neoquassin, and picrasmin would appear to be a mixture. Apart from the conversion of *iso*quassin into quassinol and except in so far as the reactions involve an oxidising agent the transformations tabulated by Clark in his memoirs must be discarded.

In a recent communication, which became available to us after the completion of the present work,* Adams and Whaley (*J. Amer. Chem. Soc.*, 1950, **72**, 375) have described the isolation of neoquassin, m. p. 229—231° and *iso*quassin, m. p. 222—225°, by a chromatographic method. Mainly with the aid of infra-red absorption spectra these authors have obtained evidence regarding the nature of the functional groups present in their compounds.

* The results contained in the present communication were described in theses submitted for the Degree of Doctor of Philisophy of this University by Dr. H. Worthington in 1940 and by Dr. E. London in 1949. A. R.

Experimental.

Isolation of the Quassin–Neoquassin Mixture.—(*a*) Powdered wood of *Quassia amara* Linn. (180 kg.) was extracted in batches (10 kg.) three times with hot water (35 l. for each batch), and the crude product was isolated from the aqueous extracts by a procedure similar to that of Clark (*loc. cit.*) and crystallised once from dilute methanol; yield, 120 g. of crystalline mixture, m. p. *ca.* 179—187°. When a solution of this solid (40 g.) in hot methanol (400 ml.) was diluted with warm water (400 ml.; 60°) and filtered through a thin layer of charcoal, the filtrate deposited a crystalline product, mainly in plates (*ca.* 20 g.), m. p. 195—198°. From the mother-liquor, which had been heated and then diluted with warm water (800 ml.; 60°), more crystalline solid separated, mainly in dense prisms, m. p. 205—209°, and then on concentration in a vacuum the aqueous-methanol liquor deposited further fractions of mixed crystals. By a prolonged fractional crystallisation (50 times) from aqueous methanol the greater part of the starting material was ultimately separated into two main fractions—(*A*) a quassin–neoquassin fraction which separated from dilute methanol in micaceous plates, m. p. 213·5°, $[\alpha]_{5461}^{20}$ +37·0° (*c*, 1·51 in chloroform), unchanged on repeated recrystallisation, and (*B*) a fraction, m.p. 219°, rich in neoquasisn.

(*b*) The charcoal containing the product absorbed from the aqueous extracts of the wood (52 kg.) was extracted in a large Soxhlet apparatus with boiling acetone, and after 15 hours the acetone extract was removed and replaced with fresh solvent. This extraction was repeated eight times (until the charcoal was exhausted) and then by a stepwise concentration of the combined extracts in a vacuum a brown crystalline product was obtained. Alternatively, evaporation of the acetone extracts in a vacuum gave an amber syrup which set to a hard mass on cooling; yield, 150—175 g. Impurities were removed from either of these products by two methods. (i) A solution of the brown solid (65 g.) in chloroform (140 ml.) was poured on a column (28 × 40 cm.) of alumina and the colourless quassin–neoquassin mixture washed through with chloroform (270—300 ml.), leaving a colourless zone at the bottom above which were narrow dark-brown, buff, brown, and light-brown zones. Concentration of the chloroform liquors gave a pale-yellow product (46 g.) which on crystallisation from ethyl acetate formed irregular plates (15 g.), m. p. 198—200°; a second crop of crystals (17 g.), m. p. 189—193°, was obtained by concentration of the ethyl acetate mother-liquor. (ii) A solution of the brown solid (45 g.) in chloroform (180 ml.) was vigorously agitated with alumina (225 g.), the mixture was filtered, the oxide was well washed with chloroform, and the combined filtrate and washings were evaporated in a vacuum, leaving an almost colourless solid (33 g.) which was crystallised from ethyl acetate.

The alumina from these experiments was retained for the extraction of a small amount of a yellow compound. Eluted with methanol a sample gave tiny amounts of a substance which formed pale-yellow needles, m. p. 250—254°, from aqueous methanol, giving a deep pink solution with aqueous sodium hydroxide and a red colour with sulphuric acid (Found: C, 57·4; H, 5·2; OMe, 27·7%).

Separation of Quassin and Neoquassin.—(a) *By chromatography.* The following is a typical example of many experiments employing this method which depends on neoquassin being slightly more strongly adsorbed than quassin on alumina. A solution of the pale-yellow or almost colourless crystalline mixture (10 g.) in chloroform (25 ml.) was poured on a column of oxide (44 cm. × 2·8 cm.) and the chromatogram developed with chloroform until the lower, very narrow, yellow-brown zone had almost reached the lower end of the column. Usually the chromatogram then consisted of a lower pale-yellow zone, a broad, almost colourless, middle zone (approx. 30 cm. in length) and an upper series of narrow yellow to brown zones. Examination of the column or photography in ultra-violet light emphasised the presence of the foregoing zones but failed to reveal any subdivision of the middle colourless zone. Similarly, staining the extruded column with a lateral streak of aqueous permanganate indicated the top and bottom zones but did not differentiate between sections of the broad colourless zone. It did, however, indicate how far the development of the quassin–neoquassin chromatogram had proceeded. The colourless section of the column was arbitrarily divided into 4 parts which were eluted with hot methanol; the lowest section gave almost pure quassin, m. p. 220°, the next two sections gave mixtures crystallising from aqueous methanol in plates and prisms, m. p. 201—203° and m. p. 208—217°, and the uppermost section gave a fraction, m. p. 220°, rich in neoquassin. By the repetition of this process with the arbitrary division of the colourless columns as indicated by experience it was possible to separate the mixture into quassin, m. p. 222°, neoquassin, m. p. 228°, and several mixed fractions; the yields of the fractions from 50 g. of mixture were respectively 10 g., 13 g., and 24 g. (which could be resolved into the 2 constituents).

(b) *By the alkali method.* A solution of the crystalline, mixed product (47·3 g.) in cold methanol (250 ml.) containing potassium hydroxide (12·5 g.) was treated with charcoal, filtered through a thin layer of kieselguhr, and diluted with water (1250 ml.). The crystalline neoquassin (12·8 g.), m. p. 219—222°, which began to separate immediately, was isolated 24 hours later and the liquor then extracted several times with chloroform (total vol., 500 ml.), giving more neoquassin (10·25 g.), m. p. 220°. On being saturated with carbon dioxide the alkaline liquor then gave a crystalline precipitate of quassin (9·2 g.) in plates, m. p. 218—220°; a further quantity (6·4 g.) was obtained by extraction of the liquor several times with chloroform (total vol., 400 ml.). From the residual aqueous liquor, which had been acidified (Congo-red) with hydrochloric acid, a brown resin (3·5 g.) was isolated with chloroform. Thus obtained, quassin and neoquassin were either purified by recrystallisation from aqueous methanol or again subjected to the methanolic potassium hydroxide treatment, followed by recrystallisation. By this process the crude mixture (47·3 g.) gave quassin (14 g.), m. p. 222°, and neoquassin (21 g.), m. p. 228°.

A solution of the brown resin (3·5 g.) in benzene was poured on to a short column of alumina. The column was well washed with benzene and then chloroform and the combined washings evaporated in a vacuum, leaving a *compound* which separated from alcohol in short needles or from benzene–light petroleum (b. p. 60—80°) in hexagonal prisms (0·43 g.), m. p. 246—248° (decomp.), giving a negative ferric reaction [Found, in a specimen dried in a high vacuum at 80°: C, 67·5; H, 6·8; OMe, 15·8%;

M, 350. $C_{19}H_{20}O_4(OMe)_2$ requires C, 67·4; H, 7·0; OMe, 16·6%; *M*, 374]. This compound, which readily forms an amorphous 2 : 4-dinitrophenylhydrazone, is easily soluble in cold chloroform or acetone, moderately soluble in benzene, ethyl acetate, or alcohol, and insoluble in aqueous sodium carbonate. It dissolves slowly in cold 2N-aqueous sodium hydroxide, forming pale-yellow solutions from which it is not precipitated by carbon dioxide.

Quassin.—Purified either by the chromatographic or the alkali method, this compound separated from aqueous methanol in transparent rectangular plates, m. p. 222°, $[\alpha]_D^{20}$ +34·5° (*c*, 5·09 in chloroform), $\lambda_{max.}$ ~255 mμ., $E_{1\,cm.}^{1\%}$ ~300, $\varepsilon_{max.}$ ~11,650, giving a negative ferric reaction in alcohol or water [Found : C, 67·9; H, 7·3; OMe, 16·6. Calc. for $C_{20}H_{22}O_4(OMe)_2$: C, 68·0; H, 7·2; OMe, 16·0%]. It is readily soluble in cold acetone, chloroform, pyridine, or acetic acid and in warm ethyl acetate, benzene, or alcohol, and sparingly soluble in ether or light petroleum. A solution of quassin in alcoholic hydrogen chloride containing 2 : 4-dinitrophenylhydrazine hydrochloride, which had been kept for 4 days and then filtered, slowly deposited an orange product. The first crop was an amorphous orange solid, m. p. 180° (decomp.) after sintering at 160°. This was followed by an orange microcrystalline *compound*, m. p. 185° (decomp.) after sintering at 164° (Found : N, 9·7. $C_{28}H_{32}O_9N_4$ requires N, 9·7%). Attempts to crystallise the crude product were unsuccessful before or after chromatography from benzene on alumina and elution with chloroform; it separated from hot alcohol as an amorphous orange solid, m. p. 198—199°. Specimens of this derivative prepared from quassin which had been isolated respectively by chromatography and by the alkali method appeared to be identical and on admixture formed a single orange zone on chromatography.

Neoquassin.—Obtained from the crude extract either by the chromatographic or the alkali method, neoquassin crystallised from aqueous methanol in thick prisms, m. p. 227·5—228·5°, $[\alpha]_D^{20}$ +41·0° (*c*, 4·98 in chloroform), $\lambda_{max.}$ ~255 mμ., $E_{1\,cm.}^{1\%}$ ~308·6, $\varepsilon_{max.}$ ~12,040, giving a negative ferric reaction [Found : C, 67·6; H, 7·8; OMe, 16·0. Calc. for $C_{20}H_{24}O_4(OMe)_2$: C, 67·7; H, 7·7; OMe, 15·9%]. In its behaviour with organic solvents neoquassin closely resembles quassin but it is in general slightly less soluble. Occasionally neoquassin separated in long, narrow plates (almost needle-shaped prisms), m. p. 213° (Found : C, 67·9; H, 7·9; OMe, 16·0%; *M*, 396), which had the same specific rotation as the prismatic form and on being kept in contact with the mother-liquor slowly changed into thick prisms, m. p. 227·5—228·5°. Slow crystallisation of neoquassin from dilute methanol appeared to favour the separation of the stable, and rapid crystallisation of the metastable, form. The most satisfactory conversion of neoquassin, m. p. 213°, into the form, m. p. 227·5—228·5°, is effected by the addition of water (3 vols.) to a solution in cold 5% methanolic potassium hydroxide. In the course of 12 hours neoquassin separates almost quantitatively in thick prisms, m. p. 227·5—228·5°.

When a solution of neoquassin (0·5 g.) in alcoholic potassium hydroxide (0·7 g. in 12·5 ml. of alcohol and 1·5 ml. of water) was heated under reflux on the steam-bath for ¾ hour and diluted with water (50 ml.), the compound, m. p. 226—227°, was quantitatively recovered.

Acetylation of neoquassin (0·25 g.) with pyridine (0·7 ml.), and acetic anhydride (1 ml.) at room temperature for six days followed by decomposition of the unchanged anhydride with ice–water gave the *acetate*, which separated from ethyl acetate–light petroleum (b. p. 60—80°) in tiny colourless prisms, m. p. 213—215° (Found : C, 66·7; H, 7·0. $C_{24}H_{32}O_7$ requires C, 66·7; H, 7·4%). This experiment sometimes failed to give the acetate, which was readily hydrolysed.

Norquassin.—Quassin (1 g.) was heated under reflux with 3·5% hydrochloric acid (60 ml.) for 1½ hours and the cooled solution, which had deposited a white crystalline solid, was partially neutralised (acid to Congo-red) with 2N-aqueous sodium hydroxide. Next day the solid, m. p. 239—240° (decomp.), was collected, well washed with water, and crystallised from aqueous methanol, giving *norquassin* in colourless parallelogram-like plates (0·56 g.), m. p. 241·5—242·5° (decomp.), $[\alpha]_{5461}^{22.5}$ +43·6° (*c*, 7·21 in chloroform) [Found, in a specimen dried in a high vacuum at 80° : C, 67·3; H, 7·0; OMe, 8·5. $C_{20}H_{23}O_5(OMe)$ requires C, 67·4; H, 7·0; OMe, 8·3%], which gave a pale red-violet ferric reaction in alcohol and on admixture with quassin had m. p. 200—220°. This compound is easily soluble in alcohol, ethyl acetate, or chloroform, sparingly soluble in benzene, and insoluble in light petroleum. It dissolves very slowly in aqueous sodium carbonate and readily in aqueous sodium hydroxide; the alkaline solution reacts with benzenediazonium chloride, giving an orange-red product. Acetylation of norquassin (0·1 g.) with acetic anhydride (0·8 ml.) and pyridine (1 ml.) at room temperature for 24 hours gave the *acetate*, which formed transparent plates, m. p. 236°, from aqueous methanol, having a negative ferric reaction (Found, in a specimen dried in a high vacuum at 80° : C, 66·3; H, 6·5. $C_{23}H_{28}O_7$ requires C, 66·3; H, 6·7%).

An excess of ethereal diazomethane was added to norquassin (0·2 g.) in methanol (18 ml.) at 0°, and three days later the solution was evaporated in a vacuum and the residue thoroughly extracted with N-aqueous sodium hydroxide, leaving an alkali-insoluble product (0·05 g.). Crystallisation of this from aqueous methanol gave quassin in characteristic rectangular plates, m. p. 221—222° undepressed on admixture with an authentic specimen (Found : C, 67·6; H, 7·3; OMe, 14·5%). On being saturated with carbon dioxide the alkaline extracts of the crude methylation product gave a compound (0·15 g.), m. p. 204°, which, on repeated crystallisation from aqueous methanol, formed colourless needles, m. p. 219·5—220·5°, readily soluble in aqueous sodium hydroxide, insoluble in aqueous sodium hydrogen carbonate, and giving a negative ferric reaction (Found : C, 67·6; H, 7·5%). This compound, which failed to react with benzenediazonium chloride, did not form an acetate, or a *p*-nitrobenzoate.

Bisnorquassin.—A mixture of quassin (0·5 g.), acetic acid (5 ml.), and concentrated hydrochloric acid (1·5 ml.) was heated on the steam-bath for one hour; after ten minutes an amber-coloured solution was formed which gradually darkened and became opaque (deep blue-green in thin layers). The hot mixture was diluted with water (6·5 ml.), treated with a little charcoal, and filtered. On cooling the pale-amber filtrate deposited bisnorquassin in short rectangular plates (0·33 g.), m. p. 263°, which on

recrystallisation from aqueous acetic acid and then ethyl acetate or alcohol had m. p. 263—264°, $[\alpha]_{5461}^{20}$ +60·6° (*c*, 0·841 in acetone), giving a pale-green ferric reaction in alcohol (Found, in a specimen dried in a high vacuum at 105° : C, 66·9; H, 6·7. Calc. for $C_{20}H_{24}O_6$: C, 66·7; H, 6·7%). This compound, which gave a deep orange-red product with benzenediazonium chloride, readily dissolved in aqueous sodium hydroxide or sodium hydrogen carbonate. With acidic 2 : 4-dinitrophenylhydrazine hydrochloride it gave a product which separated from alcohol in canary-yellow, rectangular plates, m. p. 211—212° (decomp.) (Found : C, 53·0; H, 5·9; N, 9·6. Calc. for $C_{26}H_{28}O_9N_4$: C, 47·8; H, 5·2; N, 10·4%). By the pyridine–acetic anhydride method bisnorquassin furnished the diacetate which formed colourless prisms, m. p. 232°, from alcohol, insoluble in alkali and giving a negative ferric reaction [Found, in a specimen dried in a high vacuum at 105° : C, 65·2; H, 6·5; Ac, 18·4. Calc. for $C_{20}H_{22}O_6(Ac)_2$: C, 64·9; H, 6·3; Ac, 19·4%]. Oximation of bisnorquassin (0·5 g.) with hydroxylamine hydrochloride (1 g.) and pyridine (10 ml.) on the steam-bath for two hours followed by the addition of dilute acetic acid (100 ml.) to the cooled mixture gave the *dioxime* in tiny prisms, very sparingly soluble in the usual organic solvents, forming a yellow solution in aqueous sodium hydroxide and giving a negative ferric reaction in alcohol. On the addition of methanol or alcohol to its solution in the minimum amount of pyridine at 100°, this derivative separated in colourless needles, decomposing at 308—310° (Found, in specimen dried in a high vacuum at 100° : C, 61·4; H, 6·9; N, 7·4. $C_{20}H_{26}O_6N_2$ requires C, 61·5; H, 6·7; N, 7·2%).

iso*Quassinic Acid*.—A solution of quassin (0·5 g.) in N-alcoholic potassium hydroxide (12 ml.; containing 1·5 ml. of water) was heated under reflux for ½ hour in an atmosphere of nitrogen, diluted with water, heated on the steam-bath to evaporate a part of the alcohol, cooled, and extracted with chloroform (20 ml. × 3) to remove a small amount of a neutral product. After the addition of more water (20 ml.) the solution was saturated with carbon dioxide, extracted with chloroform (3 × 20 ml.) to remove a trace of phenolic product which had a faint odour reminiscent of guaiacol, acidified (Congo-red) with concentrated hydrochloric acid, and again extracted with chloroform (3 × 20 ml). Evaporation of the dried chloroform extracts and crystallisation of the acidic residue (0·45 g.) from ethyl acetate–light petroleum (b. p. 80—100°) gave iso*quassinic acid* in colourless hexagonal prisms (0·15 g.), m. p. 206° (decomp.), having a negative ferric reaction in alcohol [Found, in a specimen dried in a high vacuum at 80° : C, 64·6; H, 7·6; OMe, 14·5%; *M* (Rast), 394; equiv. (by titration), 404, 398. $C_{20}H_{24}O_5(OMe)_2$ requires C, 65·0; H, 7·4; OMe, 15·3%; *M*, 406]. This acid is sparingly soluble in cold water and readily soluble in aqueous sodium hydrogen carbonate with the evolution of carbon dioxide. It rapidly decolorises aqueous potassium permanganate and forms an amorphous yellow-orange precipitate with acidic 2 : 4-dinitrophenylhydrazine hydrochloride but does not reduce Fehling's solution or form an azo-dye with benzenediazonium chloride. Prepared quantitatively with diazomethane, *methyl* iso*quassinate* separated from methanol in squat prisms, m. p. 180—181° [Found : C, 65·9; H, 7·8; OMe, 21·7. $C_{20}H_{23}O_4(OMe)_3$ requires C, 65·7; H, 7·6; OMe, 22·1%].

When *iso*quassinic acid was heated to 200—220° there was considerable frothing, and after ½ hour the brown residue was cooled and crystallised from aqueous methanol, giving a neutral product in needles, m. p. 264°, insoluble in water or cold 2N-aqueous sodium hydroxide.

Evaporation of the ethyl acetate–light petroleum mother-liquors from quassinic acid left a light-yellow oil. Slow spontaneous evaporation (several weeks) of a solution of this oil in the same solvent gave a second monobasic acid in colourless needles (40 mg. from 2 g. of quassin), m. p. 220—221°, which on recrystallisation, however, had m. p. 215° [Found : C, 66·0; H, 7·8%; equiv. (by titration), 442].

Dibasic Acid from Quassin.—A mixture of quassin (2 g.), N-alcoholic potassium hydroxide (48 ml.), and water (6 ml.) was heated under reflux for ½ hour, diluted with water (100 ml.), and kept at room temperature for 6 weeks. By the procedure employed in the case of quassinic acid there was isolated from this mixture a neutral product (30 mg.) and phenolic material (80 mg.), leaving an aqueous liquor which was acidified (Congo-red) and decanted from resinous acidic precipitate. From the acidic liquor chloroform (5 × 15 ml.) extracted a yellow solid which was triturated with ethyl acetate (8 ml.), giving an acid in needles (0·23 g.), m. p. 273° (decomp.). A further quantity of this acid (0·2 g.) was obtained by clearing a solution of the resinous precipitate in aqueous sodium hydrogen carbonate with a little charcoal followed by acidification and extraction with chloroform. Recrystallised from ethyl acetate, the dibasic *acid* formed colourless needles, m. p. 282—283° (decomp.), giving a negative ferric reaction and slowly forming an acidic solution in cold water [Found : C, 59·9; H, 6·4; OMe, 6·3%; equiv. (by potentiometric titration), 207; *M*, 414. $C_{18}H_{21}O_4(OMe)(CO_2H)_2$ requires C, 59·7; H, 6·2; OMe, 7·3%; equiv., 211; *M*, 422. $C_{18}H_{23}O_4(OMe)(CO_2H)_2$ requires C, 59·4; H, 6·6; OMe, 7·3%]. This acid, which gave an amorphous 2 : 4-dinitrophenylhydrazone, is soluble in hot methanol or alcohol and sparingly soluble in chloroform. Prepared with an excess of ethereal diazomethane, the *dimethyl* ester separated from aqueous methanol in colourless needles, m. p. 218°, insoluble in aqueous sodium hydrogen carbonate but readily soluble in dilute aqueous sodium hydroxide [Found : C, 61·1; H, 7·1; OMe, 20·4. $C_{20}H_{21}O_6(OMe)_3$ requires C, 61·3; H, 6·7; OMe, 20·7. $C_{20}H_{23}O_6(OMe)_3$ requires C, 61·1; H, 7·1; OMe, 20·6%].

Norneoquassin.—A mixture of neoquassin (1 g.), water (54 ml.), and concentrated hydrochloric acid (6 ml.) was gently heated under reflux for 1½ hours. Considerable frothing occurred during the early stages of the reaction but the evolution of carbon dioxide could not be detected. On being kept the cooled reaction mixture deposited *norneoquassin* in colourless prisms (0·78 g.). When the filtrate from this product was almost neutralised (acid to Congo-red) a further quantity (0·06 g.) was obtained. Recrystallised from aqueous alcohol or a little ethyl acetate, the compound formed colourless rectangular plates, m. p. 212° [Found, in a specimen dried in a high vacuum at 110° : C, 66·7; H, 7·5; OMe, 8·5. $C_{20}H_{25}O_5(OMe)$ requires C, 67·0; H, 7·5; OMe, 8·2%], soluble in aqueous sodium hydroxide but not in aqueous sodium carbonate and giving a deep violet ferric reaction in alcohol, and a red azo-dye with benzenediazonium chloride. The acetylation product was an alkali-insoluble, white amorphous solid

which could not be crystallised and regenerated norneoquassin on hydrolysis with cold 2N-aqueous alcoholic sodium hydroxide.

Norneoquassin was also prepared by heating neoquassin with 15% hydrochloric acid on the steam-bath for 1½ hours.

O-*Ethylneoquassin*.—Concentrated hydrochloric acid (2·25 ml.) was added to a solution of neoquassin (1 g.) in hot alcohol (22 ml.), and the mixture cooled immediately to room temperature, kept for four days, almost neutralised, and diluted with water (vol. of water and alkali added, 44 ml.). On being kept at 0° for one hour the mixture deposited a crystalline product (0·92 g.), m. p. 170—190°, which separated from light petroleum in irregular plates, m. p. 170—190°. Fractional crystallisation of this material from aqueous methanol followed by dilute acetic acid gave O-*ethylneoquassin* in long pointed plates, m. p. 190—192° [Found : C, 68·6; H, 8·1; OAlkyl 10·3. $C_{20}H_{23}O_3(OMe)_2(OEt)$ requires C, 68·9; H, 8·1; OAlkyl, 11·5%], along with a small amount of a second product, m. p. 201·5—202·5°, and a little unchanged neoquassin. A mixture of ethylneoquassin and the second product, m. p. 201·5—202·5°, melted at 170—182°.

It was found that repeated crystallisation of the crude ethylneoquassin from dilute acetic acid and then dilute alcohol gave a product in elongated rectangular plates, m. p. 178°, which appears to correspond to Clark's " ethoxyneoquassin " (*loc. cit.*) (Found : C, 68·5; H, 7·9%). When the alcohol was replaced by methanol O-*methylneoquassin* was obtained, forming dense prisms, m. p. 156°, from dilute methanol [Found, in a specimen dried in a high vacuum at 110° : C, 68·0; H, 8·0; OMe, 23·1. $C_{20}H_{23}O_3(OMe)_3$ requires C, 68·3; H, 7·9; OMe, 23·0%]. This compound, which closely resembled *O*-ethylneoquassin, was accompanied by a small amount of a substance which separated from the mother-liquor in long prisms, m. p. 170°.

Oxidation of Neoquassin.—A warm solution of potassium dichromate (1 g.) in acetic acid (10 ml.) was added to neoquassin (1 g.) in the same solvent (5 ml.) at about 56°, and the mixture kept for four days at room temperature, diluted with water (30 ml.), neutralised with potassium carbonate, and extracted with chloroform (5 × 65 ml.). Evaporation of the combined dried extracts left a pale-yellow residue (0·74 g.) which, on crystallisation from dilute methanol and then ethyl acetate–light petroleum (b. p. 60—80°), gave quassin, m. p. 222° undepressed on admixture with an authentic specimen, $[\alpha]_D^{20}$ +34·5° (*c*, 5·09 in chloroform) (Found : C, 67·9; H, 7·6; OMe, 14·8%). Mixed with neoquassin, it melted at 205—207°.

Hydrogenation of Quassin.—(*a*) The absorption of hydrogen by quassin (0·25 g.), dissolved in alcohol (10 ml.) containing an active Raney nickel catalyst (2 g.), ceased in about 20 minutes. On isolation the product was separated into unchanged quassin, m. p. 222°, and neoquassin, m. p. 228°, by the alkali method; yield of neoquassin, 25% of theoretical. Thus prepared, neoquassin formed characteristic thick prisms, m. p. and mixed m. p. 228°, $[\alpha]_D^{20}$ +41·4° (*c*, 1·22 in chloroform), from aqueous methanol (Found : C, 67·6; H, 7·8%). On being refluxed with 3·5% hydrochloric acid this specimen of neoquassin gave norneoquassin.

(*b*) A solution of quassin (0·5 g.) in 5% methanolic or alcoholic potassium hydroxide (5 ml.) was diluted with alcohol (5 ml.) and water (30 ml.), and agitated with hydrogen and a palladium–charcoal catalyst (from 1 g. of charcoal and 0·06 g. of palladium chloride) for one hour; approximately one mol. of hydrogen was absorbed. The filtered solution was saturated with carbon dioxide and extracted several times with chloroform. Obtained from the dried chloroform extracts, *dihydroquassin* separated from water and then alcohol in large glistening plates which contain solvent of crystallisation and, on being slowly heated, melted at 106°, resolidified, and then melted at 154° [Found, in a specimen dried in a high vacuum at 80° and then at 110° : C, 67·8; H, 7·7; OMe, 13·1. $C_{20}H_{24}O_4(OMe)_2$ requires C, 67·7; H, 7·7; OMe, 15·9%]. This compound, which behaved as a lactone, did not give a ferric reaction, form an azo-dye, or decolourise neutral or acidic aqueous potassium permanganate.

Attempted Dehydrogenation of Neoquassin.—(*a*) An intimate mixture of neoquassin (10 g.) and powdered selenium (30 g.) was kept at 220—240° for ½ hour and then at 330° for 20 hours, and extracted with boiling ether. The washed and dried extracts were evaporated and the residual brown oil distilled over a little sodium in a high vacuum, giving a pale-yellow phenolic oil (0·1 g.), b. p. 60—70°/0·2 mm., which readily crystallised. After having been drained on a tile this *phenol* was purified by sublimation in a high vacuum at 60°/0·15 mm. and obtained in colourless needles, m. p. 70°, easily soluble in aqueous sodium hydroxide and insoluble in aqueous sodium hydrogen carbonate [Found : C, 72·5; H, 8·8; OMe, 14·4. $C_9H_{11}O(OMe)$ requires C, 72·3; H, 8·4; OMe, 18·7%]. With alcoholic ferric chloride it gave a greenish-yellow coloration.

(*b*) A mixture of neoquassin (1 g.), *p*-cymene (0·6 ml.), and palladium–charcoal (Zelinsky and Pollak, *Ber.*, 1925, **58**, 1298) (0·25 g.) was heated (oil-bath at 250°) for two hours in an atmosphere of carbon dioxide. The cooled mixture was extracted with ether and then with hot benzene, and the combined extracts were washed with 2N-aqueous sodium hydroxide to remove a trace of alkali-soluble product, dried, and evaporated, leaving an orange residue which separated from a little warm alcohol as a yellow crystalline solid (65 mg.), m. p. 220—230°. A benzene solution of this product, which did not form a picrate, was poured on a column of alumina and the chromatogram developed with chloroform, giving a lower colourless zone and an upper yellow zone. Elution of the yellow zone with chloroform–methanol yielded only a trace of a yellow solid which gave a blue coloration with cold concentrated sulphuric acid changing to violet in warm acid. The colourless zone furnished a solid which was washed with a little ether and crystallised from ethyl acetate, giving a *substance* in colourless prisms, m. p. 253° [Found : C, 70·8; H, 6·9%; *M* (Rast), 352. Calc. for $C_{21}H_{14}O_5$: C, 70·8; H, 6·8%; *M*, 356].

The residue left on evaporation of the alcoholic liquor remaining after the purification of the crude dehydrogenation product was chromatographed on alumina from chloroform–benzene (1 : 3) and the column washed with benzene. Evaporation of the washings left an orange residue which on crystallis-

ation from a little alcohol gave more of the yellow solid, m. p. 220—230°, containing the foregoing compound, m. p. 253°. Addition of ether to the alcoholic filtrate precipitated a *substance* in wedge-shaped prisms, m. p. 182—183°, after sublimation [Found: C, 69·6; H, 7·8, 7·9%; *M* (Rast), 305, 361. Calc. for $C_{20}H_{26}O_5$: C, 69·3; H, 7·6%; *M*, 346].

UNIVERSITY OF LIVERPOOL.

[*Received, July* 31*st*, 1950.]