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*iso*Thiocyanates XXXIV *. The Absolute Configuration of (–)-5-Vinyl-2-oxazolidinethione (Goitrin) and its Glucosidic Progenitor (Progoitrin)

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The antithyroid factor, (–)-5-vinyl-2-oxazolidinethione (I), produced by enzymic hydrolysis of a glucoside (progoitrin) present in roots and seeds of, *e. g.*, rape and turnips, has been demonstrated to possess the absolute configuration depicted as (II). The salt of (–)-1-amino-3-buten-2-ol and (+)-α-bromocamphor-π-sulphonic acid, formerly employed for the synthesis of (I), has been transformed into the *dextro*rotatory acid oxalate of 1-amino-2-butanol.

The latter salt has also been prepared from (–)-2-hydroxybutyric acid of known absolute configuration by a sequence of reactions not involving chemical bonds attached to the asymmetric carbon atom. (+)-1-Amino-2-butanol acid oxalate has been transformed into (–)-5-ethyl-2-oxazolidinethione of known absolute configuration.

On the likely assumption that enzymic hydrolysis of progoitrin proceeds with retention of configuration the above correlation further establishes the stereochemistry of the parent glucoside.

The presence of a goitrogenic factor in roots and seeds of various cruciferous species had long been suspected at the time Astwood *et al.*[1] succeeded in isolating from yellow turnip (rutabaga) a *levo*rotatory, crystalline compound to which the structure (I) was attributed and which upon ingestion elicited goitrous symptoms in animals and human subjects. The same authors [2] presented evidence for (I) being a secondary component produced by enzymic fission of a parent substance, possibly a glycoside. This view was supported by spectroscopic evidence in our laboratory and one of the stereoisomeric 2-hydroxy-3-butenyl *iso*thiocyanates, enzymically liberated from a glucoside, was suggested as a likely progenitor of (I)[3]. A similar conclusion was reached by Schultz and Wagner [4] who isolated a crystalline tetraacetate of the parent glucoside ('glukorapiferin') from seeds of a rape variety. Shortly thereafter, Greer [5] announced the isolation of progoitrin, the crystalline sodium salt of

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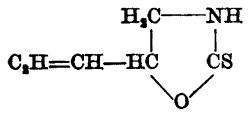
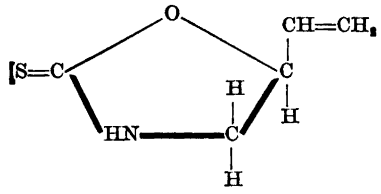
the glucosidic precursor of (I), which was enzymically hydrolyzed to glucose, sulphate and (-)-5-vinyl-2-oxazolidinethione (goitrin) *. The structure of the latter was substantiated by Ettlinger [6] who synthesized (I) by a series of reactions which left the configuration around the asymmetric carbon atom as the sole undecided structural feature. In connexion with current studies in this laboratory, knowledge of the absolute configuration of (I) became desirable. The present paper records experimental data supporting (II) as a correct expression for the *levo*rotatory 5-vinyl-2-oxazolidinethione (goitrin).

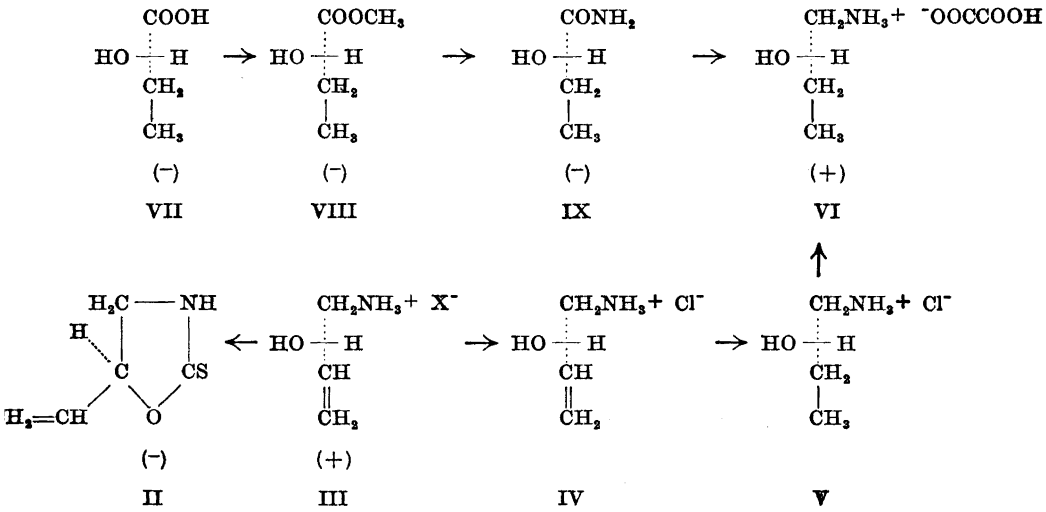


I



II

In his synthesis, Ettlinger [6] transformed the salt (III) of (-)-1-amino-3-buten-2-ol and (+)-α-bromocamphor-π-sulphonic acid into (-)-5-vinyl-2-oxazolidinethione (II). From the same salt, prepared in essentially the same way, we obtained by ion exchange the corresponding hydrochloride (IV). Catalytic hydrogenation of this afforded the hydrochloride of the corresponding 1-amino-2-butanol (V), which was, in turn, transformed into the *dextro*rotatory acid oxalate of the same amino-alcohol (VI).



* Greer, as well as Virtanen *et al.*[17], used the prefix L to denote this *levo*rotatory enantiomorph, a specification which should be dismissed in this context because of its generally accepted usage in expressing generic relationship within a particular configurational series. In the present case, however, such a correlation has not been previously established. Even if it had, the prefix would be an ambiguous or, at best, arbitrary specification.

The steric relationship of the salt (VI) was established by an alternative synthesis departing from a configurationally known compound. As such served *levo*rotatory 2-hydroxybutyric acid (VII), prepared by resolution of the racemic acid with brucine [7]. The acid was transformed into the previously unknown (-)-methyl 2-hydroxybutyrate (VIII) and thence into the more strongly *levo*rotatory 2-hydroxybutyramide (IX), which was reduced with lithium aluminium hydride to give an optically active 1-amino-2-butanol, characterized as the *dextro*rotatory acid oxalate (VI). The latter possessed the same rotation, both with regard to magnitude and sign, as the salt prepared as described above. Idential melting point, undepressed on admixture, and coinciding infra-red spectra further served to corroborate the identity of the two preparations.

The literature contains abundant evidence to support the assignment of the Fischer projection formula (VII) to the *levo*rotatory enantiomorph of 2-hydroxybutyric acid. Thus, Levene and Haller [8] correlated chemically, through the enantiomeric 2-butanols, (+)-lactic acid and (-)-2-hydroxybutyric acid.*

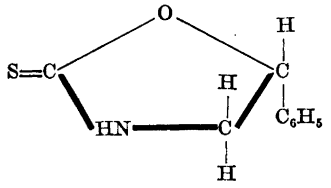
Szarvas [9] adduced good evidence for the configurational relationship of the two acids by rotatory dispersion studies, whereas Fredga *et al.*[10] demonstrated the same generic connexion by utilizing the quasi-racemate method. Supplementary evidence for (-)-2-hydroxybutyric acid being generically related to (+)-lactic acid is available by the fact that the rotations of both are similarly displaced on salt formation in accord with the general rule of Clough, which has been demonstrated to apply for monoasymmetric 2-hydroxy-substituted carboxylic acids [11]. Again, a direct correlation of (—)-hydroxybutyric acid with (—)-malic acid of ascertained configuration has been established by Horn and Pretorius [12] through an anodic coupling process.

The above correlations place the spatial representation of (—)-2-hydroxybutyric acids as (VII), and hence its absolute configuration, beyond any doubt. The same applies for the amine salt (VI) because the subsequent transformations into ester (VIII), amide (IX) and amine salt (VI) proceed without participation of bonds extending from the asymmetric carbon atom. In addition, Noyce and Denney [13] demonstrated an asymmetric center next to a carboxyl or carbonyl grouping to be unaffected by lithium aluminium hydride reduction. Again, inversion can safely be excluded in the catalytic hydrogenation and ion exchange reactions utilized in transforming the sulphonate (III) into the oxalate (VI). Although no rigorous proof is available of the mechanisms by which an optically active 2-amino-alcohol is transformed into an active 2-oxazolidinethione such as (II), there can hardly be any doubt that in the present case a base-induced cyclization of an intermediate *iso*thiocyanate represents the actual events. This further implies that no rupture of the C-O-linkage, or any other bond attached to the asymmetric center, occurs;

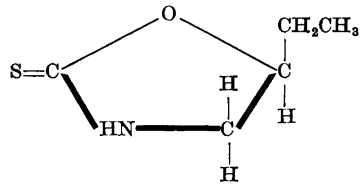
* In fact, the authors reported this acid as a *dextro*rotatory compound. Szarvas [9] subsequently demonstrated it to be slightly *levo*rotatory when measured in pure water, and further pointed out that Levene and Haller's measurement was performed in a solution of 0.58 M barium chloride and excess hydrochloric acid, resulting in an inversion of the sign of rotation. Similar conclusions were reached by other groups also [10, 12].

a conclusion which establishes the absolute configuration of goitrin (II) * relative to that of the salt (III) and, hence, of the acid (VII).

In previous communications from this laboratory the presence in seeds of *Barbarea vulgaris* R.Br.[15] and inflorescenses of *Reseda luteola* L.[16] of a glucoside (glucobarbarin) furnishing (–)-5-phenyl-2-oxazolidinethione (barbarin) upon enzymic hydrolysis, was demonstrated. Stereospecific synthesis of barbarin [16] further showed it to possess the absolute configuration depicted as (X)**



X



XI

It is interesting that goitrin and barbarin, both *levo*rotatory in methanolic solution, contain the substituents at the asymmetric carbon atom in opposite spatial positions. It hence became of interest to transform *levo*rotatory 1-amino-2-butanol acid oxalate (VI) into the corresponding 5-ethyl-2-oxazolidinethione possessing the absolute configuration (XI)***. The latter turned out to be *levo*rotatory in methanolic solution like the configurationally analogous goitrin. The ultra-violet absorption spectrum of (XI) was of the expected type and very similar to that of goitrin, a fact which supports the recent conclusion by Virtanen *et al.*[17] that (XI) can be excluded as the substance responsible for the absorption pattern observed in a milk fraction from a cow which was fed progoitrin-containing rape seed in amounts corresponding to 4.5 g of goitrin. Experimental details for the synthesis of (XI) will appear in a forthcoming communication.

The above determination of the absolute configuration of goitrin also establishes the configuration around the asymmetric carbon atom in the side-chain of progoitrin, because there can hardly be any doubt that cyclization of the intermediate 2-hydroxy-3-butenyl *iso*thiocyanate proceeds with full retention of configuration. The possible consequences of the above stereochemical studies for the biogenesis of the ring-producing *iso*thiocyanate glucosides are being considered at the present.

EXPERIMENTAL

All melting points are uncorrected and determined in capillary tubes in a slowly heated bath. Microanalyses were performed by Mr. P. Hansen at the Chemical Laboratory of the University of Copenhagen.

(±)-*1-Amino-3-buten-2-ol.* This unsaturated amino-alcohol was prepared by the route developed by Ettlinger [6] and the various synthetic reactions proceeded essentially as

* In the system for specification of absolute, asymmetric configuration proposed by Cahn *et al.*[14], goitrin (II) is (*S*)-5-vinyl-2-oxazolidinethione.

** (*R*)-5-Phenyl-2-oxazolidinethione in the specification system of Cahn *et al.*[14]

*** (*S*)-Ethyl-2-oxazolidinethione in the specification of Cahn *et al.*[14]

described. 3,4-Epoxy-1-butene, serving as the starting material, was prepared from butadiene as reported by Kadesch [18]. It was found advantageous, as proposed by Raciszewski *et al.*[19], to add a trace of pyrogallol to the 1-amino-3-buten-2-ol prior to distillation, resulting in a 74 % yield of distilled amino-alcohol based on the acid oxalate. Without polymerization inhibitor the yield was considerably lower (*cf.* also Ref.[19]) than that (79 %) reported by Ettlinger [6].

Salt of (—)-1-Amino-3-buten-2-ol and (+)-α-bromocamphor-π-sulphonic acid. Resolution of the above racemic amino-alcohol with (+)-α-bromocamphor-π-sulphonic acid (British Drug Houses) proceeded as reported by Ettlinger [6]. After recrystallization from wet ethyl acetate the monohydrate of the slightest soluble salt, m. p. 98—102°, possessed the rotation data: $[\alpha]_D^{21}$ +64° (H_2O, *c* 2.3), $[\alpha]_D^{21}$ +55° (0.5 % NaOH, *c* 2.1). A subsequent preparation, conducted at a time when the relative humidity in the laboratory was lower, resulted in isolation of the lower-melting [6], anhydrous salt.

Acid oxalate of (—)-1-amino-2-butanol (VI). The above bromocamphorsulphonate (3 g), dissolved in water (200 ml), was percolated slowly through a column containing an anion exchange resin (Amberlite IR-4B, about 120 mequiv.) in the chloride form. The combined filtrate and washing was concentrated to dryness when the hydrochloride (IV) remained as a viscous oil. Without further purification, the hydrochloride was dissolved in 96 % ethanol (20 ml), containing a few drops of water, and the solution was subjected to catalytic hydrogenation at atmospheric pressure in the presence of 10 % palladium charcoal (1 g). Hydrogen (140 ml) was rapidly taken up and after about 10 min. the absorption came to a stop. After filtration, the filtrate was concentrated *in vacuo* to an oil, consisting of (V), which was dissolved in water. The solution was made alkaline with NaOH, saturated with NaCl and subjected to continuous extraction with ether in a liquid-liquid extractor. To the ether-containing distillation flask was added oxalic acid dihydrate (950 mg) before extraction was initiated. After 3 h, the crystalline acid oxalate (500 mg) was removed by filtration. Two recrystallizations from absolute ethanol, with the use of a little charcoal, afforded a pure specimen of *dextro*rotatory acid oxalate of 1-amino-2-butanol (VI) (330 mg), m. p. 149—150°, $[\alpha]_D^{21}$ +11.3° (H_2O, *c* 0.69).

The substance showed no melting point depression on admixture with a specimen synthesized by another route described below. The infra-red spectra of the two preparations were coinciding throughout the wave-length range (650—4 000 cm^{-1}) examined.

(—)-2-Hydroxybutyric acid VII. 2-Bromobutyric acid (271 g), prepared in 76 % yield according to the directions for the synthesis of 2-bromo*iso*caproic acid [20], was transformed into (±)-2-hydroxybutyric acid in 67 % yield by the method of Bischoff and Walden [21].

The racemic acid (250 g), dissolved in water (1 300 ml), was resolved by means of brucine (966 g of anhydrous alkaloid base), as described by Guye and Jordan [7, 22]. The salt (410 g) was recrystallized from water (700 ml) to give a product (270 g), which possessed rotation values in accord with those reported [7]. To a solution of the salt in water (300 ml), conc. ammonia (50 ml) was added and the precipitated brucine was filtered off and washed. The filtrate was acidified with conc. HCl, saturated with NaCl, and subjected to continuous extraction with ether [9] for 6 h. After removal of the solvent from the dried ether solution, the crystalline (—)-2-hydroxybutyric acid remained (45 g). It was purified by distillation, b. p. 97° at 1.2 mm, and was obtained as hygroscopic, colourless needles (32 g), m. p. 53°, unchanged on recrystallization from a mixture of chloroform and pentane, $[\alpha]_D^{21}$ −15.3° (172 mg of acid in water, containing 1.3 equiv. of ammonia; total volume 5.00 ml). Literature values: m. p. 55—55.5° (recryst. from CCl_4)[10], 52.7—53.5° (from CCl_4: hexane) [12], $[\alpha]_D^{25}$ −15.9° (NH_4-salt in H_2O, *c* 2.7)[10].

(±)-Methyl 2-hydroxybutyrate. Because attempts to esterify 2-hydroxybutyric acid by the method of Clemmensen and Heitman [23] and by the customary hydrochloric acid-catalyzed procedure were unsatisfactory, due to the large amounts of by-products formed by intra- and inter-molecular condensations, special conditions were worked out with the racemic acid as a model in order to minimize the undesired side reactions. The following procedure was eventually adopted:

To a refluxing solution of anhydrous methanol (700 ml), containing dry HCl (6.7 g), was added in 7 portions, and with 0.5 h intervals, a total of 35 g of (±)-2-hydroxybutyric acid. The solution was refluxed for another 2 h when ether (100 ml) and enough water to

produce an aqueous phase of a few ml, were added, followed by solid $NaHCO_3$ until neutralization. The organic layer was removed by decantation, dried over Na_2SO_4 and freed of ether by distillation. The methyl ester distilled as a colourless liquid, b. p. 69° at 30 mm, n_D^{25} 1.4170. The yield was 19.8 g (50 %). Reppe [24] reports: b. p. 68° at 30 mm.

(−)-Methyl 2-hydroxybutyrate (VIII). The above procedure, applied to (−)-2-hydroxybutyric acid (32 g), afforded the corresponding optically active methyl ester (16.8 g) with the same b. p., n_D^{25} 1.4171, D_4^{24} 1.048, $[\alpha]_D^{24}$ −2.30° (neat). (Found: C 51.30; H 8.89. Calc. for $C_5H_{10}O_3$: C 50.85; H 8.53.)

(−)-2-Hydroxybutyramide (IX). A solution of (−)-methyl 2-hydroxybutyrate (10.0 g) in conc. aqueous ammonia (330 ml) was left standing in a closed vessel at room temperature for 3 days, whereupon it was concentrated *in vacuo* to a viscous oil. This was refluxed for one hour with sodium-dried ether (200 ml) which was decanted hot from undissolved material, and the process was repeated with a fresh portion of ether (200 ml). The combined ether solutions deposited on cooling overnight a crop of the hygroscopic amide as colourless small prisms (2.85 g). An additional crop of the same quality (2.26 g) separated from the concentrated mother liquor. An analytical specimen was secured by an additional recrystallization from anhydrous ether. Due to hygroscopicity it proved necessary to determine the m. p. in capillary tubes, sealed after drying over P_2O_5, m. p. 61−62°, $[\alpha]_D^{23}$ −45° (H_2O, *c* 1.15). (Found: C 46.65; H 8.72; N 13.40. Calc. for $C_4H_9NO_2$: C 46.60; H 8.80; N 13.59.) It is interesting to notice that the higher-melting racemic 2-hydroxybutyramide (m. p. 105° [25], 108° [26]) does not possess hygroscopic properties, a finding paralleled in the lactamide series.

Reduction of (−)-2-hydroxybutyramide to (VI). In a three-necked flask, provided with magnetic stirring, dropping funnel, nitrogen inlet and a Soxhlet-type extractor was placed a solution of lithium aluminium hydride (6.0 g) in dry ether (660 ml). (−)-2-Hydroxybutyramide (4.0 g) was placed in the extractor and washed down in the course of 2 h by the refluxing ether, during which time the reaction mixture was stirred in a slow stream of dry nitrogen. Excess reagent was then destroyed by addition of wet ether, whereafter a saturated solution (300 ml) of potassium sodium tartrate was introduced. The ether layer was combined with three 250 ml-extracts of the aqueous phase. The amine content in the dried ether solution was determined by titration to 1.21 g, corresponding to a 34 % yield.

Addition of the amine solution to an ethereal solution of oxalic acid (2.05 g) resulted in the separation of the acid oxalate in crystalline form (1.76 g), m. p. 150°. An analytical specimen was produced on recrystallization from absolute ethanol, m. p. 150°, alone or in admixture with the sample described above; $[\alpha]_D^{22}$ +11.8° (H_2O, *c* 0.71). (Found: C 40.30; H 7.27; N 7.91. Calc. for $C_6H_{13}NO_5$: C 40.21; H 7.31; N 7.82). As further criterion of identity of the two independently synthesized specimens served the coinciding infra-red spectra which, in addition to numerous expected bands, displayed an unidentified, characteristic peak at 718 cm^{-1} (KBr-disc).

Acid oxalate of (−)-1-amino-3-buten-2-ol. This salt was desired particularly for the purpose of studying its infrared spectrum. It was prepared from the above α-bromocamphor-π-sulphonate by liberation of the amino-alcohol with strong base, a process accompanied by unknown secondary reactions, followed by treatment with one equivalent of oxalic acid. The salt crystallized from absolute ethanol in colourless prisms, m. p. 134°, $[\alpha]_D^{23}$ −5.9° (H_2O, *c* 1.0). (Found: N 7.60. Calc. for $C_6H_{11}NO_5$: N 7.90). Reported m. p. for the racemic salt: 130.5−132° [6].

The infra-red spectrum, was very similar to that of the acid oxalate of the saturated amino-alcohol (VI), having in common with the latter the band at 718 cm^{-1}. Significant deviations were noted, however, particularly in the 800−1 100 cm^{-1} region.

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