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STUDY OF THE FORMATION OF THE 2,4-DICHLOROBENZENE-BORONATE DERIVATIVE OF 1-ISOPROPYLAMINOPROPAN-2-OL BY TRANSBORONATION*

EDITH SHERWOOD, COLIN F. POOLE**, WEN-FA SYE and ALBERT ZLATKIS*
Department of Chemistry, University of Houston, Houston, TX 77004 (U.S.A.)
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SUMMARY

The 1-isopropylaminopropan-2-ol group can be selectively converted to a cyclic, 2,4-dichlorobenzeneboronate derivative with good gas chromatographic properties. The reaction does not occur significantly in the solution phase under normal experimental conditions, but a quantitative yield of derivative can be formed in an on-column reaction at the point of injection by either direct reaction with the boronic acid or by transboronation. The transboronation reaction using the 2,4-dichlorobenzeneboronate derivative of catechol as the derivatizing reagent was found to be superior to the use of other reagents for derivatizing the 1-isopropylaminopropan-2-ol group.

INTRODUCTION

A common structural feature of the β-adrenoceptor blocking drugs used in the control of hypertension is the presence of a 1-isopropylaminopropan-2-ol side-chain which is substituted in the 1-position by a phenoxy group. The side-chain group contains a bifunctional structural unit (*i.e.* contains two protonic functional groups in close proximity) which should be capable of forming a cyclic derivative with an appropriately substituted reagent[1]. In an earlier investigation, it was shown that the β-blocking drug alprenolol formed a cyclic 2,4-dichlorobenzeneboronate derivative which enabled low levels of the drug to be determined in biological fluids after extraction into an organic solvent and gas chromatography with electron-capture detection[2]. Unlike previous studies employing 2,4-dichlorobenzeneboronic acid as a selective derivatizing reagent of bifunctional groups, no evidence could be obtained for a reaction occurring in solution under the usual experimental conditions. The balance of probability favored an on-column reaction occurring at the point of injection. However, the precision obtained by direct boronation (reaction between the 2,4-di-

* Determination of bifunctional compounds, Part XI. For Part VIII, see ref. 2.
** Present address: Department of Chemistry, Wayne State University, Detroit, MI 48202, U.S.A.

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chlorobenzeneboronic acid and the bifunctional group of the drug) was poor and could not be used with the electron-capture detector due to saturation of the detector by the large excess of boronic acid required to convert the 1-isopropylaminopropan-2-ol group to its 2,4-dichlorobenzeneboronate derivative. To circumvent this problem, a new reaction (transboronation) was developed in which co-injection of the β-blocking drug with the 2,4-dichlorobenzeneboronate of 1,3-propanediamine was used to derivatize the side-chain group and to provide a volatile reagent which elutes rapidly from the column causing the minimum of detector disturbance. The transboronation reagent used in the above study was not ideal for all β-blocking drugs as some secondary peaks resulting from either impurities in the transboronation reagent or from thermal decomposition of the reagent at the high temperatures used to volatilize the drugs, interfered in the chromatography of some of the β-blocking drugs and also resulted in a high detector background signal.

The transboronation reaction is a potentially very useful reaction for the derivatization of the side-chain in the β-blocking drugs. In the light of the shortcomings of the original transboronation reagent we have investigated the properties of several new transboronation reagents. As the nature of the reaction and the parameters of importance in obtaining a quantitative yield of derivative needed further clarification we have investigated the influence of the experimental variables on the yield of derivative using the model compound 1-isopropylaminopropan-2-ol.

EXPERIMENTAL

1,3-Propanediamine, 1,3-propanediol, 1,3-aminopropanol, 1,4-butanediol, *o*-aminophenol, catechol and phenanthracene* were obtained from Aldrich (Milwaukee, WI, U.S.A.), The 1-isopropylaminopropan-2-ol (H 170/37) and metoprolol were a gift from Dr. J. Vessman (AB Hässle, Mölndal, Sweden). 2,4-Dichlorobenzeneboronic acid was obtained from Lancaster Synthesis (St. Leonardgate, Lancaster, Great Britain) or from the Alfa Products Division (Ventron Corporation, Danvers, MA, U.S.A.).

For studies of the reaction mechanism, a solution of 1-isopropylaminopropan-2-ol was prepared by dissolving 58.51 mg (0.5 m*M*) of the compound and 44.56 mg (0.25 m*M*) of the internal standard, phenanthracene in methylene chloride (5.0 ml). In a typical reaction, 50 μl (5.2 μ*M*) of the above solution was added to the appropriate amount of either 2,4-dichlorobenzeneboronic acid or transboronation reagent and sufficient methylene chloride added for complete solution. From 1 to 4 μl of the above mixture was used for analysis.

For gas chromatography, a Perkin-Elmer 3920 gas chromatograph with an on-column injector and flame ionization detectors was used. For analysis, a 3 ft. × 0.2 cm I.D. glass column packed with 2% OV-225 on Gas-Chrom Q (100–120 mesh) was used. The analytical conditions used were varied widely to suit the individual problem. The optimum conditions for transboronation of 1-isopropylaminopropan-2-ol using catechol 2,4-dichlorobenzeneboronate (CDCBB) were: injection temperature, 190°C; column temperature, 165°C; nitrogen carrier gas flow-rate, 25 ml min^{-1}. Under these conditions, the retention times were: for the 2,4-dichlorobenzeneboronate

* Also known as phenanthrene.

of 1-isopropylaminopropan-2-ol, 3.0 min; for phenanthracene, 7.0 min; for the 2,4-dichlorobenzeneboronate of catechol, 16.0 min.

RESULTS AND DISCUSSION

The peak area observed for the 2,4-dichlorobenzeneboronate derivative of 1-isopropylaminopropan-2-ol was found to be dependent on the excess molar concentration of the boronic acid (up to the point where the reaction is complete) but was independent of the reaction time (0–8 h) and reaction temperature (20–100°C) in the solution phase. From the above observations, we concluded that the boronate reaction occurred essentially at the point of injection onto the column and that the reaction in solution was by comparison unimportant. Similar observations were made in the preparation of the 2,4-dichlorobenzeneboronate derivative of alprenolol[1]. Derivatization of the 1-isopropylaminopropan-2-ol group was complete when co-injected with a *ca.* 15 *M* excess of 2,4-dichlorobenzeneboronic acid (Fig. 1). This compares with a *ca.* 30 *M* excess needed for the reaction with alprenolol[2]. However, the on-column boronation reaction is unsuitable for general use as the build-up of excess reagent on the column both affects the stability of the detector baseline and eventually leads to the production of derivative peaks of poor shape. To obtain reproducible results, it is necessary to clean the column of excess boronic acid between injections. This is best achieved by injecting in sequence 1–2 μl of 1,3-propanediol, 1 μl of silyl 8 and 10 μl of Freon 112 at the normal column operating temperature for the analysis.

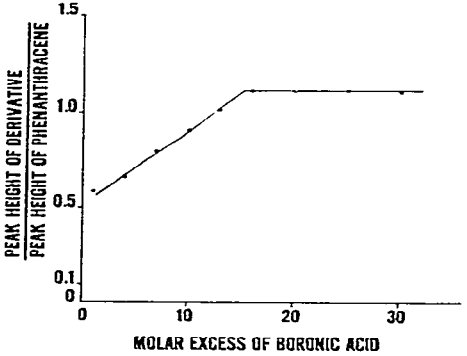


Fig. 1. The formation of the 2,4-dichlorobenzeneboronate derivative of 1-isopropylaminopropan-2-ol by boronation under optimum conditions.

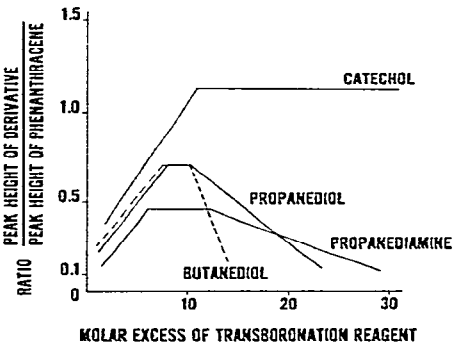


Fig. 2. A comparison of transboronation reagents for the derivatization of 1-isopropylaminopropan-2-ol.

To overcome the problem of carrying out an on-column reaction with a non-volatile reagent, the technique of transboronation was developed[2]. For this reaction, the 1,3-propanediamine derivative of 2,4-dichlorobenzeneboronic acid was selected. The general stability of this derivative is moderate and it was considered that it would most likely react favorably with the 1-isopropylaminopropan-2-ol group under the conditions employed for the transboronation reaction. In practice, certain shortcomings were found with this reagent. Extraneous peaks due either to impurities in the

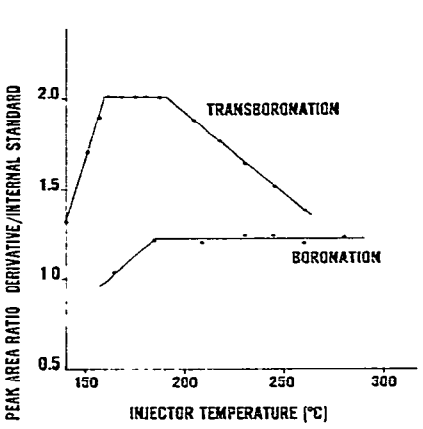
transboronation reagent or by-products resulting from thermal decomposition of the reagent interfered with the derivatives of some of the β-blocking drugs. To identify a more useful reagent for the reaction, the properties of several new transboronating reagents were investigated. As can be seen from Fig. 2, different results were obtained depending on the transboronation reagent evaluated. The 2,4-dichlorobenzeneboronate derivative of 1,3-aminopropanol produced a very low yield of 1-isopropylaminopropan-2-ol derivative and was considered to be unsuitable for use as a transboronation reagent. Returning to Fig. 2, it is noticeable that the plateau region for the transboronation reaction occurs at a different height for each reagent, although the amount of 1-isopropylaminopropan-2-ol and internal standard (phenanthracene) was the same for all experiments. Of the reagents tested, the catechol 2,4-dichlorobenzeneboronate reagent (CDCBB) gave the highest conversion ratio for the derivative. In this case the plateau region occurs at the same position as was observed by direct co-injection with excess boronic acid. If this plateau region corresponds to 100% conversion of the 1-isopropylaminopropan-2-ol group to its 2,4-dichlorobenzeneboronate, then for transboronation using 1,3-propanediol, 1,4-butanediol and 1,3-propanediamine derivatives of 2,4-dichlorobenzeneboronic acid, the plateau region corresponds to a yield of 63, 63 and 43%, respectively. The reason for the rapid decline in yield of derivative with increasing concentration of the transboronation reagent is unknown at present. As the highest yield of derivative was obtained with the catechol reagent and there was no decrease in the yield of derivative with increasing transboronation reagent concentration, this reagent was selected for the evaluation of the effect of the experimental variables on derivative yield. A similar series of experiments by direct boronation using co-injection of 2,4-dichlorobenzeneboronic acid were also performed for the purpose of comparison.

The effect of injection temperature on the yield of derivative

All experiments were performed using an on-column injector in which the tip of the syringe needle just reached the surface of the column packing. The remaining portion of the column was packed with silanized glass wool. As can be seen from Fig. 3, the effect of injection temperature on the yield of derivative was different for the two methods of derivative formation. For direct boronation, provided that the injection temperature exceeded 185°C, there was no temperature dependence on the yield of derivative in the range 185–290°C. The choice of injection temperature was more critical for the CDCBB transboronation reagent. The yield of derivative was a maximum for the temperature range 160–190°C, with a lower yield outside this range. An injection temperature of 185°C was selected as the optimum value for both methods of derivative preparation.

The effect of column temperature on the yield of derivative

The yield of derivative by direct boronation was independent of the column temperature in the range 140–190°C (the extent of the accessible temperature range giving reasonable retention times for the derivative). For transboronation, the yield of derivative was a maximum over the temperature range of 160–167°C (Fig. 4). At higher column temperatures, the yield of derivative was reduced. This must be related to the nature of the transboronation reaction and is not due to thermal decomposition of the derivative as boronation does not show a similar temperature dependence.



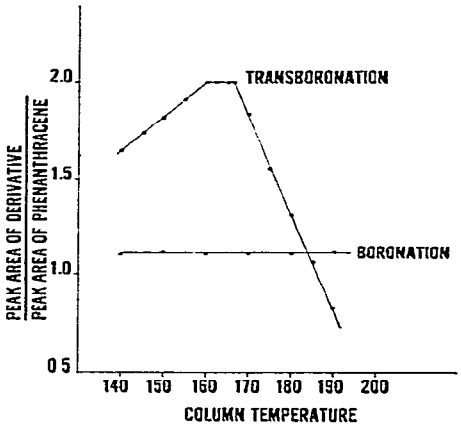


Fig. 3. The effect of injection port temperature on the yield of derivative by boronation and transboronation.

Fig. 4. The effect of column temperature on the yield of derivative by boronation and transboronation.

The effect of carrier gas flow-rate on the yield of derivative

There was no carrier gas flow-rate dependence for the boronation reaction in the range 20–50 ml min^{-1}. For transboronation, the maximum yield of derivative was obtained with a flow-rate in the range 20–30 ml min^{-1} (Fig. 5). At higher flow-rates the yield of derivative is considerably reduced.

The influence of carrier gas flow-rate and injection and column temperature on the yield of derivative by transboronation is most probably related to the dynamics of the mixing process responsible for maintaining contact between the reagent and substrate and is less likely to be due to thermal instability. If the latter was the case then a similar derivative profile would be expected for boronation as observed for transboronation.

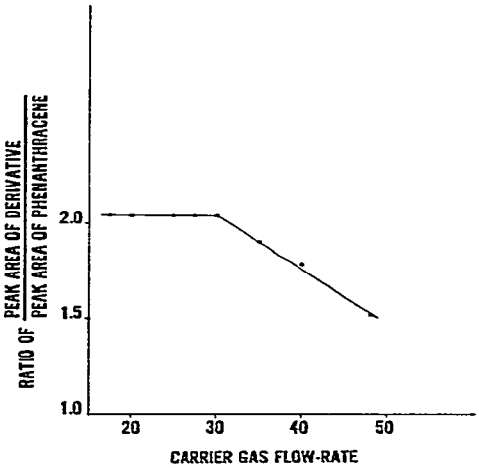


Fig. 5. The effect of carrier gas flow-rate on the yield of derivative by transboronation.

Calibration curve for the transboronation reaction

A linear calibration curve covering the range 10–150 μg of 1-isopropylaminopropan-2-ol was obtained by transboronation. The ratio of the concentration of the transboronation reagent to 1-isopropylaminopropan-2-ol was maintained at greater than 15 (*i.e.* in the plateau region for derivative formation under optimum conditions) over the range investigated. The calibration curve was reproducible, showed no significant deviation from linearity and passed through the origin.

An application of the transboronation reaction to the derivatization of metoprolol

The β-blocking drug metoprolol can be quantitatively and reproducibly converted to its 2,4-dichlorobenzeneboronate derivative by the transboronation reaction. This is the preferable method of derivatization as direct boronation is difficult to reproduce with good precision and has a deleterious influence on the chromatographic performance of the column unless excess reagent is removed from the column between injections. For metoprolol, the plateau region for derivatization was obtained with a *ca* .15 *M* excess of transboronation reagent (CDCBB). The operating conditions were: column temperature, 220°C; injection temperature, 250°C; carrier gas flow-rate, 25 ml min^{-1}; using the same column as employed for the 1-isopropylaminopropan-2-ol derivative. The change in experimental conditions compared with those employed for the derivatization of 1-isopropylaminopropan-2-ol was dictated by the difference in volatility between the two compounds. Work is in progress with metoprolol and other β-blocking drugs to evaluate the use of the transboronation reaction for their analysis in biological fluids with an electron-capture detector.

CONCLUSIONS

The transboronation reaction offers distinct advantages over direct boronation for the formation of the 2,4-dichlorobenzeneboronate derivative of 1-isopropylaminopropan-2-ol. The most useful transboronation reagent identified was the 2,4-dichlorobenzeneboronate derivative of catechol. The transboronation reaction occurs on-column at the point of injection and the yield of derivative obtained is influenced by the injection temperature, column temperature and carrier gas flow-rate. Under optimum conditions a quantitative yield of derivative is obtained in a simple, reproducible and instantaneous reaction. Compared to boronation, the reproducibility of the derivative reaction is much better and the need to clean the column between injections is eliminated.

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