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Derivatization: Tool In Analytical Separations Of Compound

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ABSTRACT

Therapeutic agents are derived from natural products and their derivatives. Drug discovery is a scientific task developing effective and new drug compounds. Derivatization is a process which allows convertion of a chemical compound into a new compound i.e., derivative which have similar structure. There are several methods, of derivatization, which can be typically used for enhancing the method sensitivity and better separation is relevant for particular compounds, classes of compounds and derivatization reagents. Some derivatives also show moderate or significant activity. An attempt has been made in this review to explain in detail various derivatization reactions like reactions in which alkyl or aryl derivatives are formed, acylation, silylation, alkylation, oxidation with respect to its mechanism and application.

keywords: Alkylation, Aryl derivatives, Chromatography, Derivatization, Oxidation, Silylation.

Introduction

Drug discovery is a scientific process that involves the development of new and effective medications. Natural products constituent and their derivatives act as a therapeutic agents to treat various ailments. Derivatization is the process of altering a compound to create a new one.[1] There is a modification of certain functional groups of reacting substances in derivatization. Simple steps to be conducted to improve the derivatization process are modifying the pH, adding proton donors or acceptors, changing the media, and adding catalysts. Some of the methods of derivatization reactions includes Acylation, silylation, alkylation, and oxidation.[2] The derivatization method is typically chosen based on the desired analyte characteristic as well as the analyte's reactivity. As a result, the reagent must have moieties that add the desired attribute to the analyte, as well as the ability to react with the functional group of the analyte. Sometimes pre-sample treatment like drying the original sample, or more sophisticated method are required to make the sample initial matrix suitable for derivatization.[3] Various derivatizations reactions are discussed which would enable the researcher to get knowledge about preparation of various types of derivatives and its application in analytical detection, also biological activities of derivatized compounds can be evaluated.

Reactions With Formation of Alkyl or Aryl Derivatives

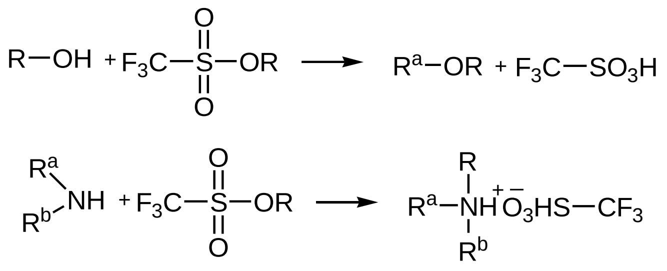
Alkyl (R) or aryl (Ar) derivatives are generated by substituting the active hydrogens of analyte with an alkyl (R) or aryl (Ar) group. COOH, OH, NH, SH and CONH are all functional groups that can be substituted. Alkyl bromides (Short-chain) or iodides derivatives of steroids, catecholamines, amino acids, phenols, sulfonamides, organic acids, barbiturates, and mono- and oligosaccharides can be formed and all have several analytical applications. A huge number of reagents R-X are known, wherein R represents a specific characteristic and X represents a specific reactivity. Both the R and X components of a molecule influence the reagent's reactivity. The type of reactive group X and the type of moiety R guide the selecting process for choosing a reagent for a particular derivatization. The analyte serves as a nucleophile (Y: Y:H, Y:-) in most alkylation reactions, reacts with the alkylating reagent R-X in a substitution (SN) reaction, which has a leaving group X and an alkyl group R [4]

Y:H + R –X Y:H + R –X Y – R + X:H

Depending on the analyte and the method, some derivatizations can be sluggish and inefficient. For analytical purposes, the reaction rate becomes a key parameter. For example, the production of alkyl (methyl or ethyl) substituents with an alkyl halide is frequently done with a particular methylation reagent, a catalyst, or in some cases with a specific solvent. A variety of different approaches can be used to improve the alkylation efficiency. For example, particular cryptands like crown ethers can be utilized to solvate the alkali metal component of an organic acid salt, which generates the free anion makes a faster nucleophilic attack. Phase transfer alkylation is another method for increasing alkylation efficiency. This approach works by creating an easily extractable chemical in an organic phase and shifting equilibrium in the direction of the desired product's development.[4] Use of various alkylating reagents such as Pentafluorobenzyl bromide (an extremely reactive halogen) is one technique to improve alkylation efficiency. This reagent can be used to derivatize a wide range of active hydrogen-containing molecules. 2-

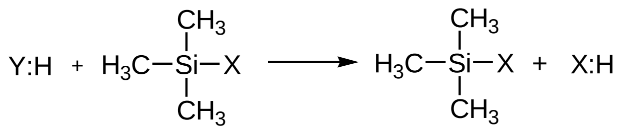
bromoacetophenone is another reactive halide (phenacyl bromide). This reagent is mostly used to alkylate compounds like carboxylic acids that contain more acidic hydrogens. Carbohydrates are another example of methylation utilizing the specific reagent R-X. The Methyl sulfonyl-methanide anion is used in this methylation.

The methyl sulfinyl-methanide anion can easily methylate a polyol or monosaccharide dissolved in DMSO. Other alkylating reagents (different X in R-X) that react in a nucleophilic substitution are also known. Alkylation’s may also be done with dimethyl sulphate, for e.g., Alkylfluoromethyl-sulfonates are far more reactive than sulphates and the reaction can also happen with active hydrogen from alcohols or amines, as shown below.



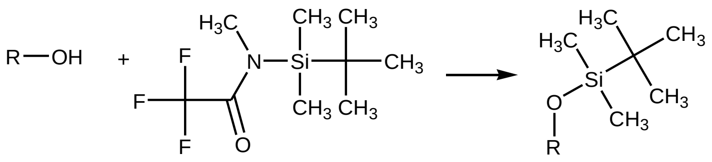
Silylation Reactions [1]

Silylation is a reaction in which a reactive hydrogen atom of functionalities like COOH, OH, SH, CONH, NH, SOH, POH, or carbonyl(enolisable) is replaced with a silyl group, oftenly trimethylsilyl (TMS), as shown below



Silylation is a most commonly used derivatization method. applicable to make a wide range of compounds. Silylation of alcohols, carbohydrates, phenols, amines, and sterols, is used in a variety of analytical methods. In chromatography, silylation can be utilized to decrease the polarity, increase stability, and improve GC behaviorr of analyte. The mass spectra of silylated compounds and that of initial analyte differs and that could also be a factor, beneficial in terms of detection. Silylation reactions can be applied for complex samples like plant material and individual isolated analytes as well. The method's biggest drawback is its vulnerability to moisture. Moisture causes a reduction in reaction yield and instability in the derivatized analytes. Traditional mass spectral libraries may not contain silylated compounds mass spectra for many compounds. Furthermore, some forms of stationary phases, such as those seen in polyethylene glycol-type columns, may be caused by silylated substances in combination with the typically present excess of silylating reagent. deteriorate over time, making separation impossible

When MTBSTFA reacts with polar functional groups containing an active hydrogen, it produces tertbutyl dimethyl silyl (TBDMS) derivatives. [5]

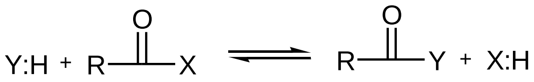


MTBSTF **TBDMS**

The detection of amino acid derivatives was aided by spectral data derived from the peaks. Formation of multiple derivatives of amino acids including asparagine, glutamine, and tryptophan can be prevented by changing the reaction conditions, such as lowering the temperature or changing the reaction time. The completely derivatized form of tryptophan has a higher response. TBDMS derivatives are more stable than typical TMS derivatives, but their higher molecular weights cause longer GC elution times. The separation was carried out on a small, narrow bore capillary column to counteract this.

Acylation Reactions

acyl derivatives are formed by replacing the active hydrogens from functionalities such as OH, NH, CONH, SH and others. Acylation reactions are mostly nucleophilic substitution reactions, in which the analyte (Y, Y:H, Y) reacts with the acylating agent RCO-X, where X is a leaving group and RCO: is an acyl group. The reaction is shown below



$$Y{:}H + R\overset{O}{\overset{\|}{C}}X \rightleftharpoons R\overset{O}{\overset{\|}{C}}Y + X{:}H$$

The acylation technique can also be used to improve the performance of analytes in chromatographic columns by reducing polarity. The analytes can be more volatile if they are acylated. Acylation of fluorinated compounds is commonly used in GC with ECD or NCI-MS detection to improve detectability. Only acetyl or fluorinated acyl groups (lighter than heptafluorobutyric) increase volatility, but other acyl groups (heavier) are ineffective. Monosaccharides and polysaccharides, for example, may be acetylated. Chiral compounds can also be isolated in better way using acylation reacttions. Various "X" groups may be added to the acyl groups in the reagent. OH is one of these classes, and some free acids are among the acylating reagents.[5] Esterification reaction occurs when the nucleophile is an alcohol. Apart from alcohols, acylation with acids can be used on thiols, phenols, amines, and other compounds, as shown below:

H + R -COOH - R - COY + H_2O Y : H + R -COOH - R - COY + H_2O

By removing the waterusing compounds such as anhydrous $MgSO_4$ or substances that react with water, such as CaC_2 or $(CH_3)_2C$, the reaction can be shifted toward the formation of acyl derivatives (OCH_3) .[6]

Derivatization of Monoterpenes

Acylation process is applicable to derivatization of monoterpenes like acyclic, monocyclic, bicyclic monoterpene derivatives E.g., Geraniol, Acyclic Monoterpene derivatives shows antimicrobial, antitumor, antioxidant, anti-inflammatory, cardioprotective, hepatoprotective and neuroprotective activities. Thymol Monoterpene monocyclic derivatives is a natural phenolic monoterpenoid It has antimicrobial and anti-inflammatory properties, making it ideal for wound healing, as well as antioxidative and anticancer properties[7]. It also shows antiviral (anti-HIV), anticonvulsant, antidepressant and antipyretic properties Myrtenal, monoterpene bicyclic derivatives is a monoterpenes made up of two isoprene molecules.[8] They have antioxidant, anticancer, and anti-diabetic properties, as well as cyclooxygenase inhibitory and immunostimulant properties[9]

Oxidation Reactions

The derivatization of compound is done by several methods and from that oxidation is one of them The capacity of a drug to function as an oxidant governs the oxidation reaction. It is a simple and cost-effective spectrophotometric approach for determining. Oxidation reaction can be applied for derivatization of beta pinene.,[10]

A hydroboration reaction converted -pinene to myrtanol, which was then oxidized to myrtanyl [11] acid by an oxidation reaction. Then, using the substructure splicing principle, a sequence of amide and acyl thiourea derivatives were made by splicing an amide or acyl thiourea moiety onto the pinane skeleton [12]

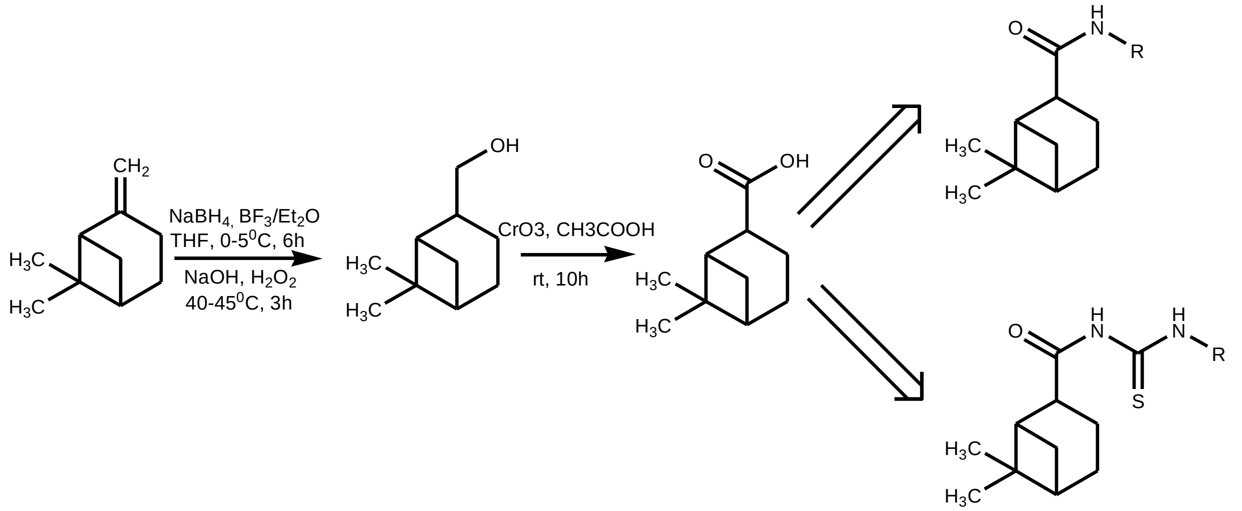


Figure 1. synthesis of beta pinene based derivatives

The antifungal activity of β -pinene-based derivatives was evaluated against five plant diseases. The acyl thiourea and amide derivative of β-Pinene showed better antifungal activity. Most of the β pinene-based derivatives displayed antifungal efficacy against Phomopsis species. [37]

Other Derivatization Reaction

- Other derivatization reactions have been employed for GC and GC/MS studies. Adding hetero multiple bonds to functional groups like CO, CS, CN, or CN is one of them.
- Aldehydes and ketones can be reacted with active hydrogens in groups such as OH, H_2N-NH-, NH_2, and so on.[13]
- Alcohols creates hemiacetals or acetals with aldehydes and ketals with ketones respectively. Stable cyclic acetals , ketals formed after derivatization, are used for analysis. [14]
- Carbonyl compounds frequently react with amines, resulting in a Schiff base or substituted imine. The use of dinitrophenylhydrazine (DNPH) in the derivatization of carbonyl compounds is a common. Liquid chromatography or gas chromatography can be used to evaluate the derivatized molecule.[15] The carbonyl compounds react with several additional types of chemicals that are comparable to hydrazine's.

- Hydrazones (NH_2NCR_2), hydrazides (NH_2NHCOR), and semi carbazides are among them ($NH_2NH-CONH_2$).[10] Oxime contains an active hydrogen when the reaction is done with hydroxylamine. This can be further derivatized by silylation with a typical silylation reagent, for example. Other reagents, such as substituted methoxy amine like hydroxylamine hydrochloride, can be employed for derivatization.
- In GC chromatographic separations, oximes in sin- and anti- forms can yield multiple peaks. Oximes, in the presence of CH_3COONa, reacts with acetic anhydride and transformed to nitriles to avoid this effect.[11]
- The conversion of oximes to nitriles results in the creation of a single product from the two (syn- and anti-) isomers,[13] which can be utilized to simplify the chromatograms of sugars which are derivatized to oximes. Other hetero multiple bonds can be reacted by alcohols, amines, and thiols, resulting in analytical applications.
- The isocyanates (NCO), the CO group in a nitrile, amide, CS_2, or other groupings The addition of alcohols to dimethyl formamide under particular conditions is one example[41]. As with N, N-dimethylformamide dimethyl acetal, the resultant acetals are highly reactive and can be employed as reagents. Another example is the creation of xanthates when CS_2 reacts with alcoholism the presence of a base. Amines react with CS_2 as well, and the resulting isothiocyanate can be studied by G.C. Derivatization is also used in sample processing to convert noncyclic chemicals to new cycles or to replace old cycles with new more stable cycles with desired feature.
- For example, Epoxides, can be produced by reacting a carbon–carbon double bonding molecule with a peroxy acid. Among the peracetic, performic, per benzoic, trifluoro per acetic, and 3,5-dinitroperoxybenzoic acids are the most often utilized peroxy acids for the production of epoxides. Epoxide separation may be easier than olefin separation, and this sort of derivatization has been used to improve the separation of various cis and trans unsaturated fatty esters. Amino acids combine with phenyl isothiocyanate to generate a thiohydantoin derivative, which is another reaction that results in the production of new cycles. This technique has been used to analyses amino acids in proteins satisfactorily. In a similar procedure, p-Bromo phenyl isothiocyanate was utilized. In reactions involving bifunctional molecules, a variety of aromatic cycles can be generated.

- Formaldehyde, for example, can combine with tryptophan or tryptamine to produce a -carboline derivative. Further derivatization by silylation of the carboxyl group, the resulting molecule can be examined by GC. The interaction of hydrazine's with diketones such as 2,4-pentandione is a common route to pyrazoles (acetylacetone). A GC separation can be used to analyses the resultant chemical. Difunctional compounds have been reported to react with activated carbonyl groups, such as those found in hexafluoroacetone. The appropriate analytes for GC and GC/MS studies can be preared by various types of derivatization processes. Among these are the production of a variety of cyclic forms of Compounds such as, boronates, siliconized, azines, and others that are thermo-stable and lack polar hydrogens, allowing for GC or GC/MS study.
- Oxidation and reduction were occasionally used for addition of reagents that added specific moieties to the analytes.
- Derivatization of sugar phosphate is done by two step derivatizations, Compounds containing a free carbonyl group, such as glucose-6-phosphate, fructose-6-phosphate, and 3-phosphoglyceraldehyde, were reacted with methoxy amine to form methoxime derivatives (CH_3ON), which were subsequently esterified using propionic acid anhydride and N-methyl imidazole as a catalyst propionylation groups.
- Compounds which lacks carbonyl group, such as glucose 1-phosphate or sucrose 6-phosphate, only reacted with propionic acid anhydride, not with methoxylamine.
- Identification, separation and quantification of sugar phosphates can be increased by ultra-high performance liquid chromatography–electrospray ionization. HPLC—MS is a mass spectrometry technique. [12] In general, By making polar molecules more hydrophobic, Oximation and propionylation of sugar phosphates is an efficient approach for increasing the retention and separation of sugar phosphates in RP-LC.[12]

Conclusion

In this study, several methods of derivatizations are discussed, such as alkylation, acylation, oxidation and some other derivatization reactions. Now a day's derivatization has become a potential tool in analytical detection of compound. the information regarding derivatization would help researcher to overcome difficulties faced during analytical detection of compound, also some of the derivative show moderate to potent activity. In conclusion derivatization techniques can be used as an important tool in GC/MS detection and other analytical methods.

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Conflict of Interests

The authors report no declarations of conflict of interest regarding publication of this article

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