COMBINATORIAL CHEMISTRY IN THE UNDERGRADUATE LABORATORY

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ABSTRACT

A practical is presented to familiarize the undergraduate students with a basic concept of the combinatorial chemistry. Using two different phenols as nucleophiles and dichloromethane as electrophile in basic medium, the mixtures of three possible substitution products are obtained which are easily separable. *[African Journal of Chemical Education—AJCE 14(1), January 2024]*

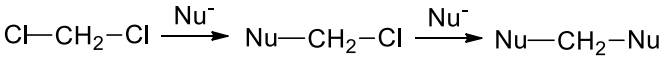
INTRODUCTION

The concept of the combinatorial chemistry was first reported by Hungarian chemist Árpád Furka approximately 40 years ago.[1] Briefly, the concept refers to obtention of the mixtures of compounds for screening rather than synthesizing and screening them one-by-one. The concept gained ample acceptance as illustrated by a number of over 8900 hits in the Web of Science as of September 2023, using a search term “combinatorial chemistry”. In addition, specialized publication venues like the ACS Combinatorial Science e.g., were launched. The mixtures of compounds obtained dubbed “libraries” are then subjected to screening to identify the active compound and to decipher its structure (“deconvolution”). Various strategies are known for this purpose and are discussed in the dedicated literature. Such approach caught attention of the pharmaceutical industry to speed-up a search for new drugs and indeed, the specialized companies offer the libraries of many thousands of compounds commercially.

RESULTS

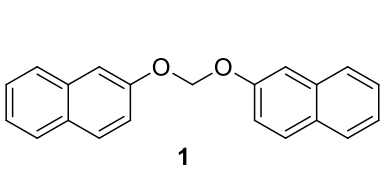
The objective of the present note is to show that a concept of the combinatorial chemistry can be easily implemented in the undergraduate laboratory as a practical, using simple and available reagents.

Dichloromethane is a common solvent, but it can be treated as a reagent due to the presence of two chlorine atoms, which can act as the leaving groups as shown in the Scheme 1. Dibromomethane, diiodomethane and bromochloromethane can be used for the same purpose, but considering a factor of price, CH_2Cl_2 is the easiest electrophile to be used.

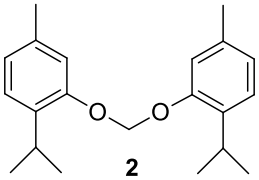


Scheme 1. Double nucleophilic substitution in CH_2Cl_2

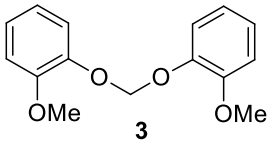
Such substitutions gained some attention and the compounds **1-3** have been prepared in basic media by geminal substitution in CH_2Cl_2 using 2-naphthol, thymol and guaiacol, respectively, as nucleophiles.



2-Naphthol formaldehyde acetal [2]

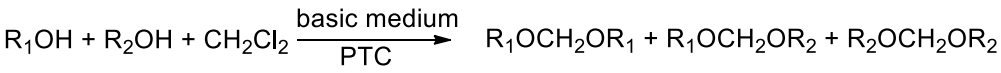


Thymol formaldehyde acetal [3]



Guaiacol formaldehyde acetal [4]

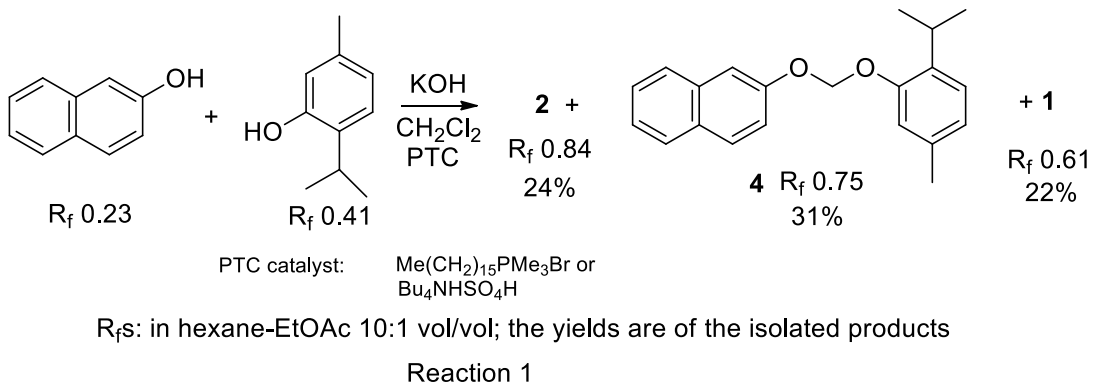
We reasoned that if two different phenols R_1OH and R_2OH were used simultaneously, a mixture of three possible products should be formed as shown in the Scheme 2.



PTC: phase-transfer catalysis

Scheme 2. Simultaneous formation of three different products: "mini-combinatorial" process

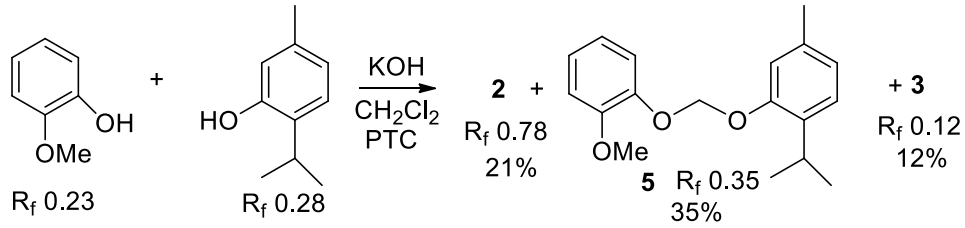
Obviously, a use of more nucleophiles would result in formation of all possible combination of the acetals. For example, the use of three phenols would result in formation of six different products. Such mixtures would be difficult to separate without the High-Pressure Liquid Chromatography setup unavailable in the undergraduate laboratories. It happens thought, that a use of combinations of two phenols only enables one to separate the products formed by simple gravitational column chromatography. Three phenols: 2-naphthol, thymol and guaiacol are the objective of the present communication since the mixtures formed accordingly to the Scheme 3 and the Scheme 4 using combinations of 2-naphthol-thymol and thymol-guaiacol, respectively, are easily separable as judged by the R_fs values of the products.



R_fs: in hexane-EtOAc 10:1 vol/vol; the yields are of the isolated products

Reaction 1

Scheme 3. Simultaneous formation of three different separable formaldehyde acetals **1**, **2** and **4**: illustration of principles of combinatorial chemistry.

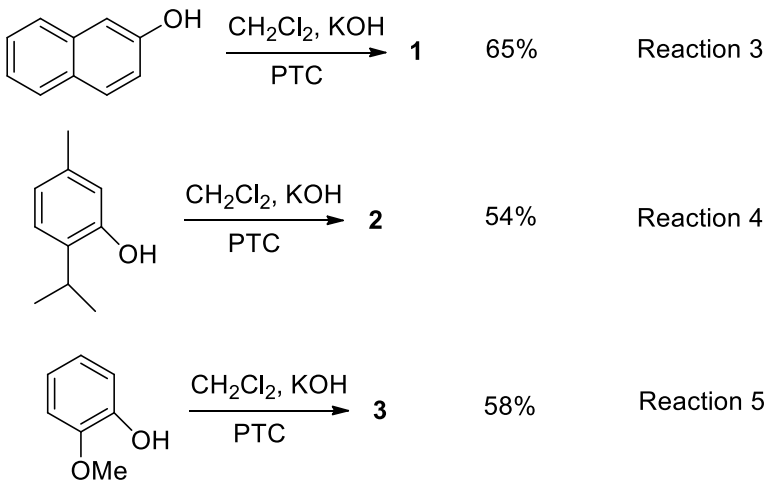


R_fs: in hexane-EtOAc 15:1 vol/vol; the yields are of isolated products

Reaction 2

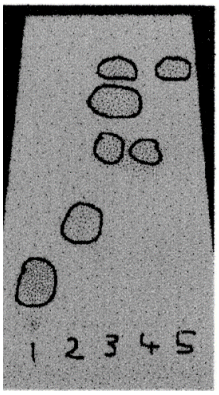
Scheme 4. Simultaneous formation of three different separable formaldehyde acetals **2**, **3** and **5**: illustration of principles of combinatorial chemistry.

The equimolar quantities of the reagents are simply incubated in dichloromethane solution with crushed KOH and the phase-transfer catalyst with or without magnetic stirring. In the latter case, the amount of KOH must be bigger, and the reaction times are longer. In one case, we left such mixture for one month without detrimental formation of the by-products. This illustrates the effectiveness of the procedure. In most cases when stirring is applied, the reactions are terminated overnight, but this is unpredictable and contingent upon the intensity of stirring and a degree of pulverization of KOH. If the unreacted substrates were still present, more PTC catalyst and more KOH should be added, and the reaction continued. The known reference compounds, the symmetrical acetals of formaldehyde, **1**,**2** and **3**, were prepared as shown in the Scheme 5 by the same procedures.

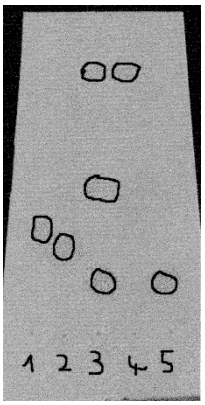


Scheme 5. Synthesis of symmetrical acetals of formaldehyde **1**,**2** and **3** by geminal substitution in CH_2Cl_2, as the reference compounds.

The TLC profiles of the two processes in the Scheme 3 (Reaction 1) and in the Scheme 4 (Reaction 2) are shown in the Picture 1 and the Picture 2, respectively.



Picture 1. TLC profile (hexane-EtOAc 10:0.5 vol/vol) of the Reaction 1, line 3; 2-naphthol, line 1; thymol, line 2; Reaction 3, line 4; Reaction 4, line 5. The spots are visualized under the UV light.



Picture2. TLC profile (hexane-EtOAc 10:0.5 vol/vol) of the Reaction 2, line 3; thymol, line 1; guaiacol, line 2; Reaction 4, line 4, Reaction 5, line 5. The spots are visualized under UV light.

It is clearly seen that the two phenols reacted completely to form the mixture of three products as expected: two symmetrical acetals and one mixed acetal which shows intermediate

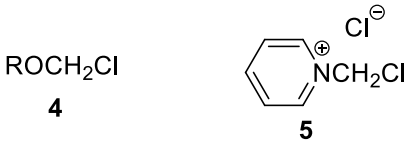
mobility on TLC. The identity of the symmetrical acetals is established by comparison of their TLC mobilities with these of the known compounds **1**, **2** and **3** obtained as shown in the Scheme 5.

At this point a basic objective of the present practical has been realized: it was shown that two different substrates form simultaneously three possible products. A time frame to conduct these experiments is as follow. To set-up of each of the alquilation one needs 1 hour. A TLC analysis of each of the reactions also requires 1 hour. This part of the procedure can be used as a basic training for the undergraduate students.

Contingent upon the conditions of a given laboratory and a level of training of the students, this practical can be continued and isolation and identification of the symmetrical and mixed acetals can be conducted. In such case 1 hour should be reserved for aqueous work-up, 6 hours for column chromatography including preparation of the silica gel column, TLC analysis of the fractions, and finally some 3 hours for crystallization and drying of the pure compounds. The students who are conversant with extraction, vacuum filtration, column chromatography and crystallization can follow this part of the procedure. The analysis of the NMR and the MS spectra is reserved for the advanced students.

CONCLUSIONS

The following points of didactic interest should be stressed. Dichloromethane is a common solvent, but it can also be treated as a reagent to realize geminal nucleophilic substitution to furnish acetals. Likewise, benzylidene dibromide, [5] benzylidene dichloride [6] and 1-dichloromethylnaphthalene [6] also furnished acetals in basic medium. In fact, chloroform with three chlorine atoms undergoes a triple substitution and obtention of triethyl orthoformate using sodium ethoxide was published. [7] Using phenols which are more nucleophilic than alcohols, one can easily obtain formaldehyde acetals in basic medium using CH_2Cl_2 even though such acetals are normally obtained in the acidic conditions from trioxane or paraformaldehyde and alcohols. A success of such double substitution is guaranteed by the impossibility of the E2 eliminations, which undoubtedly would take place with the other geminal halides, like 2,2-dihalopropanes. In addition, reactivity of the intermediate alkoxychloromethane **4** is evidently much greater than this of dichloromethane, so there is no accumulation of it by analogy to the reaction of pyridine with dichloromethane. In the latter case the reaction is slow, but a second substitution in the intermediate **5** was calculated to be ca 10000 faster than in dichloromethane. [8]



4 5

EXPERIMENTAL PART

General

All reagents were used as such without any purification. Dichloromethane was of technical grade. TLC plates coated with silica gel with a 254 nm fluorescent indicator were from Fluka. The silica gel 60 0.06 - 0.2 mm, 60 Å, for the column chromatography was from Acros. The melting points are uncorrected. The NMR spectra were recorded using Varian spectrometer at 300 MHz in $CDCl_3$ solutions. The exact mass spectra were recorded on an Exactive Plus HCD, Thermo Scientific.

General procedure to get simultaneously three acetals in a combinatorial mode as shown in the Scheme 3 and the Scheme 4.

Equimolar quantities of 2-naphthol and thymol, 2 eq. of each in 40-50 ml of CH_2Cl_2, pulverized KOH (ca 1-2g) and the phase transfer catalyst (ca 0.3g; hexadecyltrimethylphosphonium bromide or tetrabutylammonium hydrogen sulfate) were magnetically stirred overnight in a round bottom flask. KOH was crushed in a mortar under protecting layer of hexane considering highly hygroscopic nature of KOH. The hexane was

pipetted out after crushing and the resulting slurry was transferred to the reaction flask with a spatula. The amount of the KOH used is only approximate. Alternatively, 7-8g of crushed KOH was used without stirring. In this case, the mixture was left for seven or more days with occasional manual swirling. The thin layer chromatography profiles are shown in the Picture 1. The KOH was removed by filtration through a sintered glass funnel and the solids were washed with CH_2Cl_2. The combined organic phases were washed with water, dried ($MgSO_4$) and evaporated. The compound **1** can crystallize already at this stage and can by filtered out with the aid of a little volume of the hexane- EtOAc 5:1 vol/vol to furnish pure **1**. The solvents should be evaporated and the residual oil should be solubilized in a minimum volume of EtOAc – hexane 1:1 with warming if necessary and applied on top of the chromatography column prepared with Silica gel 60 (ca 100 g) and hexane-EtOAc mixture 40:1 vol/vol, and eluted initially with the same system until the less polar **2** and **4** come out. Then, the elution should be continued with hexane-EtOAc 30:1 vol/vol to get more **1**. The fractions which contain pure **2**, **4** and **1** eluted in this order as judged by TLC are pooled together and evaporated. The intermediate mixed fractions which might have been obtained were not purified further. The yields are shown in the Scheme 3. Instead of separating all three compounds, it is simpler to isolate only the least polar two products, which come out first. It is because the most polar compound **1**, the known naphthol formaldehyde acetal, can be isolated as

a crystalline compound before chromatography. Also, it can be easily prepared as shown in the Scheme 5 (see below).

Using the same procedure, guaiacol and thymol reacted in a combinatorial mode to form **2**, **5** and **3** eluted in this order using hexane-EtOAc 19:1 to get **2** and **5**, followed by hexane-EtOAc 15:1 to get **3**. The yields are given in the Scheme 4.

The most polar **3** can be obtained easier by reaction of guaiacol and dichloromethane as shown in the Scheme 5, Reaction 5.

General procedure to get symmetrical acetals of formaldehyde by double substitution in CH_2Cl_2 are shown in the Scheme 5

The phenol of choice 2-3 mmols is solubilized in dichloromethane (ca 40 ml) and the phase transfer catalyst is added (0.2-0.5 g) followed by KOH crushed under hexane (2-7 g) as shown above. The mixture is left overnight under magnetic stirring or left idle with only occasional manual swirling until all the substrate disappears (TLC). The aqueous work-up and evaporation of the solvent furnishes crystalline mass already at this stage. The crystalline products can be obtained by trituration with hexane, filtration and drying. In this way **1**, **2** and **3** can be easily prepared without chromatographic purification.

The analytical data of the products obtained are shown below

1 2-naphthol formaldehyde acetal: mp. 137-138° (hexane - EtOAc); lit. [9]: mp. 133-134°.

^{1}H: 7.77-7.25 (5 groups of signals, 14h, H aromatic), 5.94 (s, 2H, OC**H$_2$**O).

^{13}C: 154.7, 134.3, 129.6, 127.6, 127.1, 126.4, 124.3, 118.9, 110.5, 91.4 (O**C**H$_2$O)

2 thymol formaldehyde acetal: mp. 49-50° (hexane – EtOAc); lit. [10]: mp. not mentioned.

^{1}H: 7.12-6.80 (3 groups of signals, 6H, H aromatic), 5.74 (s, 2H, O-C**H$_2$**-O), 3.29 (m of 7 lines, J=6.5Hz, 2H, C**H**Me$_2$), 2.31 (s, 6H, Ph**Me**), 1.18 (d, J=6.5Hz, 12H, CH**Me$_2$**).

^{13}C: 154.2, 136.4, 134.8, 126.0, 122.9, 115.4, 91.6 (OCH$_2$O) 26.6 (Ph**Me**), 22.9 (CH**Me$_2$**), 21.2 (**C**HMe$_2$).

3 guaiacol formaldehyde acetal: mp. 90-91° (hexane – EtOAc); lit.: two articles [11, 12] do not mention about the mp.

^{1}H: 7.12-6.80 (three groups of signals, 8H), 5.77 (s, 2H, OC**H$_2$**O), 3.80 (s, 6H, O**Me**).

^{13}C: 150.1, 146.2, 123.3, 120.9, 116.0, 112.2, 93.3 (0**C**H$_2$O), 55.8 (O**Me**).

4 2-naphthol thymol formaldehyde acetal: syrup.

^{1}H: 7.94-7.01 (6 groups of signals, 10H, H aromatic). 5.99 (s, 2H, OC**H$_2$**O), 3.54 (m of 7 lines, J=7 Hz, C**H**Me$_2$), 2.52 (s, 3H, Ph**Me**), 1.39 (d, J=7 Hz, CH**Me$_2$**).

13C: 154.9, 154.1, 136.5, 134.9, 134.3, 129.6, 129.4, 127.6, 127.0, 126.3, 126.1, 124.1, 123.2, 118.9, 116.2, 110.3, 91.4 (O**C**H_2O), 26.5 (Ph**Me**), 22.9 (CH**Me$_2$**), 21.2 (**C**HMe_2).

HRMS: Cal. for $C_{21}H_{22}O_2 + Na^+$=329.1512; found: 329.1504.

5 guaiacol thymol formaldehyde acetal: syrup.

^{1}H: 7.29-6.88 (5 groups of signals, 7H, H aromatic), 5.83 (s, 2H, OC**H$_2$**O), 3.91 (s, 3H, O**Me**), 3.34 (m of 7 lines, J=7.0Hz, 1H, C**H**Me_2), 2.39 (s, 3H, Ph**Me**), 1.23 (d, J=7 Hz, 6H, CH**Me$_2$**).

^{13}C: 154.1, 150.0, 146.2, 136.4, 134.6, 125.9, 123.2, 122.9, 120.8, 117.7, 115.1, 112.0, 92.2 (O**C**H_2O), 55.7 (O**Me**), 26.4 (Ph**Me**), 22.8 (CH**Me$_2$**), 21.2 (**C**HMe_2)

HRMS: cal. for $C_{18}H_{22}O_3 + Na^+$= 309.1451; found: 309.1455.

REFERENCES

1. Furka, A. *Drug Disc.Today*, **2022**, *27*(1), 1-12.
2. Pawar, N.S., Garud, S.I., Mahulikar, P.P. *Asian J.Biochem.Pharm.Res*. **2012**, *2*(3), 157-161; CAN 158:573120.
3. Kumbhar, P.P., Kapadi, U.R., Hundiwale, D.G., Attarde, S.B., Dewang, P.M., Pawar, N.S. *Org.Prep.Proc.Int*., **2000**, *32*(6), 600-603.
4. Fujita, H.Y., *Nippon Kagaku Kaishi*, **1975**, 331. *CAN* 83:42948.
5. Damager, I., Numao, S., Chen, H., Brayer, G.D., Withers, S.G. *Carbohydr.Res*., **2004**, *339*(10), 1727-1737.
6. Tanimoto, S., Iwata, S., Imanishi, T., Okano, M. *Bull.Inst.Chem.Res., Kyoto Univ*., **1978**, *56* (3), 101-103
7. Kaufmann, W.E., Dreger, E.E. *Org.Synth*., **1925**, *5*, 55.
8. Rudine, A.B., Walter, M.G., Wamser, C.C. *J.Org.Chem*., **2010**, *75*(12), 4292-4295.

9. Tanimoto, S., Taniyasu, R., Okano, M. *J.Synth.Org.Chem, Jpn*., *Yuki Gosei Kagaku Kyokaishi*, **1971**, *29*(2), 166-168
10. More, D.H, Pawar, N.S., Dewang, P.M., Patil, S.L., Mahulikar, P.P., *Russ.J.Gen.Chem*., **2004**, *74*(2), 217-218
11. Loubinoux, B., Coudert, G., Guillaumet, G. *Tetrahedron Lett*., **1981**, *22*(21), 1973-1976.
12. Markin, G.V., Ketkov, S.Yu., Lopatin, M.A., Kuropatov, V.A., Shavyrin, A.S., Belikov, A.A., *Russ.Chem.Bull., Int.Ed*., **2020**, *69*(4), 751-757.