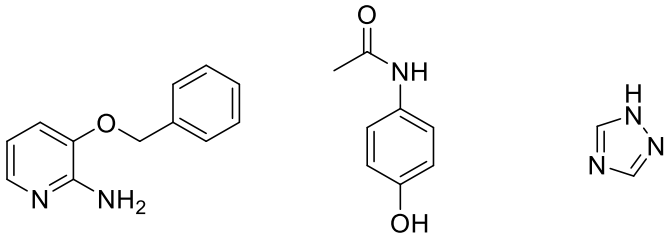
Organic chemistry practical course

Analysis

3-benzyloxy-2-pyridinamine, *N*-acetyl-4-aminophenol and 1,2,4-triazole

($C_{12}H_{12}N_2O$, $C_8H_9NO_2$, $C_2H_3N_3$)



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Characterization and seperation process

The analyte, containing three different substances, had a dark green appearance. To separate the analyte sample, column chromatography was used. Thin layer chromatography (TLC) was used to determine the adequate solvent proportion of the eluent used for the separation process. As mobile phase a mixture of PE : EE was used.

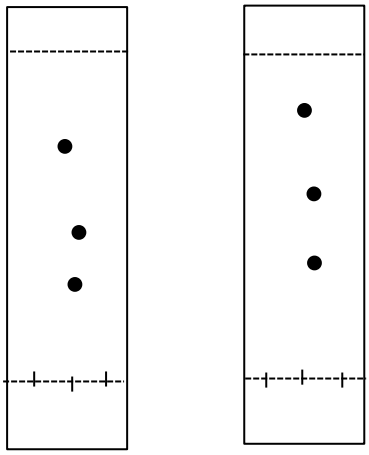

Figure 1: TLC of the analyte in PE:EE with ratio 2:1 and 1:1

On TLC (Figure 1), three spots are observed from solvent proportion PE:EE 2:1 which shows a maximal R_f value of 0.6. However, the separating capacity is not sufficient for the isolation of the three substances. Using PE:EE in a proportion of 1:1, a higher R_f value is obtained and this provides a better separation of the analyte. For this reason, the PE:EE 1:1 is used for the chromatographic separation. During column chromatography process, the first fraction was identified by spotting a small portion of the dissolved substance on the TLC plate and exposing it to UV-light, under which this spot was clearly seen. The second fraction could not be identified in the same way. In this case, the substance was coloured to blue during the irradiation with UV light. As such the second fraction could be isolated. Though the TLC analysis showed three separated fractions, the third substance in column couldn't be isolated. For the first fraction, a white solid substance was obtained after the removal of the eluent, the second fraction resembled a blue solid.

Analysis

First chromatographic separation (column chromatography) of the unknown substance took place, NMR analysis of the three obtained unknown substances was carried out for structural elucidation afterwards. For this purpose, a ^{1}H-spectrum, ^{13}C-spectrum, and DEPT-spectrum have been measured (see appendix).

Going forward, following abbreviations are used to describe the multiplicity of the ^{1}H-NMR peaks: s = singlet, d = doublet, dd = doublet of a doublet, t = triplet, m = multiplet. The DEPT-135 method provides information about the signal multiplicity in ^{13}C-spectra. Hence, a (+) describes a methyl- or methine carbon atom, a (-) describes a methylene carbon atom and (o) describes a quaternary carbon atom.

Substance 1

The ^{13}C-spectrum shows a signal around 77 ppm, which can be attributed to solvent $CDCl_3$.

A small intensity signal at 7,26 ppm of the ^{1}H-spectrum is also attributed to the solvent.

The following signals results are obtained from the spectra of substance 1.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.67 (m, 1H), 7.42-7.34 (m, 5H), 6.95 (m, 1H), 6.57 (m, 1H), 5.05 (s, 2H), 4.79 (s, 2H) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 150.4 (o), 141.5 (o), 139.1 (+), 136.4 (o), 128.7 (+), 128.3 (+), 127.6 (+), 116.8 (+), 113.6 (+), 70.2 (-) ppm.

The ^{1}H analysis shows many peak signals appearing in the aromatic region, which implies a poly-aromatic molecule. Looking at the DEPT-spectrum, at least six CH-groups and three quaternary carbon atoms are identified.

The peaks at 127.6 and 128.7 ppm have a higher or stronger intensity compared to other peaks. Therefore, the assumption is made that they belong to many equivalent C-atoms. Aditionally, an aliphatic C-atom is found, which belongs to a secondary C-atom.

4.79 (s, 2H) ppm: This signal, which is broad in appearance, is attributed to two equivalent H-atoms. Broadened Peaks arise due hydrogen bonding intermolecular forces, that is why the signal could be attributed to a O-H or N-H bond. The chemical shift as well as the fact that two protons are found suggests an amino group.

5.05 (s, 2H) ppm: This signal is not in the aromatic region, and it belongs to two equivalent protons. The narrow peak indicates the hydrogen is bonded to a carbon and it is in the vicinity of an electron withdrawing group, hence this signal is shifted downfield. This indicates the proton is attached to a secondary aliphatic carbon atom which appears in the ^{13}C-spectrum at 70.2 ppm.

6.57 (m, 1H), 6.95 (dd, 1H), 7.67 (dd, 1H) ppm: These signals appear shifted downfield, this range is characteristic to aromatic protons due to the deshielding effect of the aromatic ring current. The splitting of the signal as a doublet of doublets supports this assumption. Two hydrogen atoms appear as doublet of doublets, indicating they have a tertiary and quaternary atom in their neighbourhood. Beside this, a multiplet appears showing evidence of many hydrogen atoms in the neighbourhood, since splitting of signals can be observed over many bonds. These signals correspond to the aromatic CH-groups in the ^{13}C-spectrum. The CH-group which is split up as a multiplet is assumed to be located between the CH-group that appears as a doublet of doublets. In vicinity to the methine groups that appear as a doublet of doublets, a quarternary carbon atom must be located. In between the quaternary atoms there must be another quaternary carbon atom, otherwise the presence of another proton signal in the ^{1}H-spectrum would be observed. From the above, the following basic structure of compound 1 can be derived (**Figure 2**):

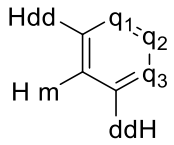

Figure 2: Structure of the aromatic system for substance 1

7.42-7.34 (m, 5H): This multiplet can be attributed to five protons. Since there is an overlapping of signals, a clear signal splitting is not observed. Nevertheless, due to the strong coupling between the protons, it can be stated these protons belong to an aromatic system. This is confirmed by the CH-signals in the ^{13}C-spectrum. Thus it can be concluded that a phenyl ring is present since this aromatic system contains five CH-groups.

Together with the other aromatic system, this gives eight aromatic methine groups. This matches with the ^{13}C-spectrum, where there are six aromatic CH-signals. Two of them are attributed to signals of strong intensity, yet three quaternary C-atoms as well as an aliphatic CH_2-group remain.

Considering the discussions above, significant peaks have been identified and the unknown compound can be identified *via* literature research utilizing the spectral database SDBS[2]. Comparing the found NMR data, only 3-Benzyloxy-2,3-pyridinamine qualifies as substance 1 because of the corresponding number of aromatic tertiary and quaternary C-atoms as well as an aliphatic CH_2-group. The measured spectra of compound 1 are matching with spectroscopic data reported in literature of 3-Benzyloxy-2-pyridinamine, confirming the assumed structure.[source of literature spectra] The NMR signals are assigned as following:

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.67 (dd, 1H, $^3J_{H,H}$ = 5.2 Hz, $^4J_{H,H}$ = 1.2 Hz, 5-H), 7.42-7.34 (m, 5H, 7-H, 8-H, 9-H, 10-H, 11-H), 6.95 (dd, 1H, $^3J_{H,H}$ = 7.8 Hz, $^4J_{H,H}$ = 1.6 Hz, 3-H), 6,57 (m, 1H,4-H), 5.05 (s, 2H, 12-H), 4.79 (s, 2H, NH_2) ppm.
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 150.4 (o, 1-C), 141.5 (o, 2-C), 139.1 (+, 5-C), 136.4 (o, 6-C), 128.7 (+, 8-C, 10-C), 128.3 (+, 9-C), 127.6 (+, 7-C, 11-C), 116.8 (+, 3-C), 113.6 (+, 4-C), 70.2 (-, 12-C) ppm.

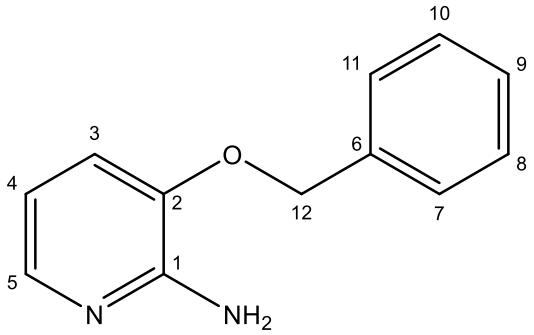

Figure 3: Numbered structure of 3-Benzyloxy-2-pyridinamine for signal assignment.

Substance 2

First, the signals at 2.50 ppm in the ^{1}H-NMR spectrum as well as the peak at 45.0 ppm in the ^{13}C-NMR can be assigned to solvent residual peaks of undeuterated DMSO. Since DMSO is hygroscopic, a residual water peak around 3.33 ppm also is expected. The spectrum shows a peak at around 3.41 ppm, which is assigned to said residual H_2O. Further, the spectra presents following NMR signals:

^{1}H-NMR (400 MHz, (DMSO-*d6*): δ = 9.64 (s, 1H), 9.31 (s, 1H), 7.34 (d, 2H), 6.68 (d, 2H), 1.98 (s, 3H) ppm.

^{13}C-NMR (100 MHz, (DMSO-*d6*): δ = 172.8 (o), 158.4 (o), 136.3 (o), 126.1 (+), 120.2 (+), 29.0 (+) ppm.

First, the ^{13}C-spectrum is considered, which shows six signals. The signal at 29.0 ppm can be assigned to an aliphatic carbon atom, as this upfield shift is characteristic for them. In the DEPT-spectrum, it appears as a positive signal. Therefore, substance 2 contains an aliphatic methyl or methine group.
The downfield shift of the four peak signals between 120.2 ppm and 158.4 ppm is characteristic for aromatic C-atoms. The aromatic C-atoms at 120.2 ppm and 126.1 ppm appear as positive signals in the DEPT spectrum and thus resemble CH - groups. Since the other two C-atoms (136.3 ppm, 158.4 ppm) do not appear in the DEPT spectrum, they are quaternary C-atoms.
Shifted even further downfield at 172.8 ppm, the last remaining signal is attributed to a carbonyl group. This premise is further validated, as the DEPT confirms it to be a quaternary carbon atom.
By interpreting the ^{13}C-spectrum, a skeletal structure with at least two quaternary C-atoms and two tertiary C-atoms is revealed. Furthermore, a carbonyl functional group with a quaternary C-atom as well as an aliphatic group with a primary or tertiary C-atom must be present.

6.68 (d, 2H), 7.34 (d, 2H): On the ^{1}H-spectrum, a significant roof effect is observed in the aromatic region, hence the presence of an AB-system is to be expected. Since the peak signals appear as doublets, the neighbourhood consists of a tertiary and quaternary atoms according to the n+1 rule. By peak integration, these doublets can be assigned to two equivalents H-atoms. Hypothesizing two different substituents, an aromatic AB-system suggests substitution in the para position. Since two quaternary C-atoms are found in the aromatic shift area, the ^{13}C-NMR data support this assumption. Further information can be derived from the intensity of the aromatic CH-groups in the ^{13}C-spectra: since their intensity is significantly higher than other peaks located in the aromatic shift area, their signal presumably originates from several, chemically equivalent, CH-groups. Therefore, substance 2 is suspected to contain following basic structure (Figure 4).

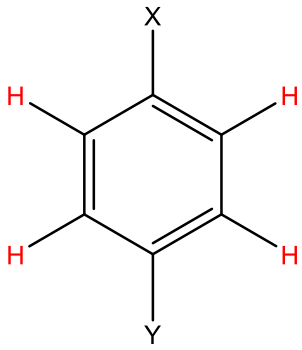

Figure 4: skeletal structure of substance 2

9.31 (s, 1H), 9.64 (s, 1H) ppm: Noticeably, two peak signals are strongly shifted downfield. Strong electron withdrawing, therefore deshielding, groups are the only possible source of such shift. Nitrogen- or oxygen-containing groups with -M and/or -I effect are in these case possibilities. Both protons appear as singlets and possess quaternary atoms in their neighbourhood.

1.98 (s, 3H): Moreover, the spectrum shows a singlet in the upfield shift area. Integration shows this signal results from three protons, hinting to a methyl group. From its multiplicity it is learned no other protons are located in proximity. The shift of 1.98 ppm is fairly downfield for an aliphatic methyl group, so an electron withdrawing group in its vicinity is probable. As already learned from the ^{13}C-spectrum, the molecule contains a carbonyl group and a quaternary C-atom. Since the carbonyl-C-atom is quaternary, an aldehyde functional group can't be present. In addition, carboxylic acids and esters would cause a stronger shift toward downfield and in conclusion are excluded. Thus, the methyl group must be connected to the carbonyl group.

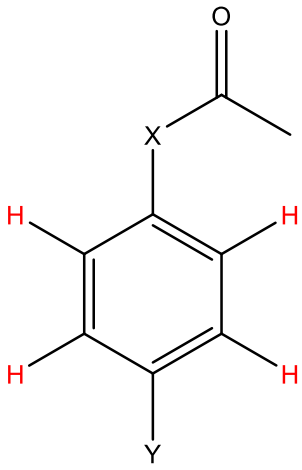

Figure 5: Basic structure for substance 2 with a ketone

So far, all the ^{13}C-NMR-signals have been assigned to carbon atoms contained in the predicted structure, to either the aromatic moiety or the carbonyl group. The remaining two singlets found in the proton NMR at a downfield shift therefore must be bound to heteroatoms. As mentioned, oxygen or nitrogen atoms, with their electron withdrawing abilities, are generally assumable. Since both signals each describe a single proton, an amino group (NH_2) can be ruled out. In the Y-position, an alcohol group is a logical solution. The multiplicity and intensity of the signal are fitting; at 9.13 ppm it also shows the characteristic shift of a hydroxy group proton due to the deshielding effect of the oxygen atom. Concerning the X-position, presence of oxygen is impossible, since X must be already bound twice to carbon atoms. An oxygen atom therefore could not bear another proton. On the other hand, nitrogen would appear as a secondary amine due to its five valence electrons, which is fitting. The signal at 9.64 ppm in the proton NMR resembles the hydrogen atom of an amide group. Concluding from all aforementioned information, substance 2 is proposed to be *N*-acetyl-4-aminophenol. NMR signals are assigned as following (Figure 6).

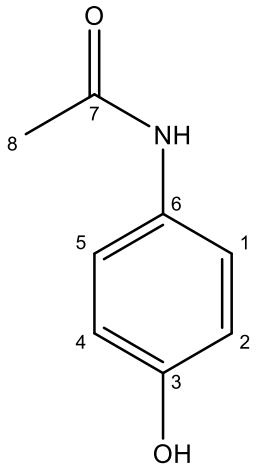

Figure 6: Signal assignment for *N*-acetyl-4-aminophenol

^{1}H-NMR (400 MHz, (DMSO-*d6*)): δ = 9.64 (s, 1H, NH), 9.31 (s, 1H, OH), 7.34 (d, 2H, ^{3}J = 8.8 Hz, 1-H, 5-H), 6.68 (d, 2H, ^{3}J = 8.8 Hz, 2-H, 4-H), 1.98 (s, 3H, 8-H) ppm.
^{13}C-NMR (100 MHz, (DMSO-*d6*)): δ = 172.8 (o, 7-C), 158.4 (o, 3-C), 136.3 (o, 6-C), 126.1 (+, 1-C, 5-C), 120.2 (+, 3-C, 5-C), 29.0 (+, 8-C) ppm.

The measured NMR spectra match with those reported in literature.[2]

Substance 3

Signals at 39.7 ppm (^{13}C-NMR) as well as 2.50 ppm (^{1}H-NMR) are assigned to residual peaks of the solvent DMSO. [1]
Furthermore, following signals were obtained from the spectra of the unknown substance:

^{1}H-NMR (400 MHz, DMSO-*d6*): δ = 8.29 (s, 2H), 14.04 (s, 1H) ppm.
^{13}C-NMR (100 MHZ, DMSO-*d6*): δ = 146.8 (+) ppm.

146.8 (+): First, the carbon NMR is interpreted. The signal found resides in the aromatic shift area and it can as such be assigned to an aromatic carbon. Since a positive signal is seen in DEPT, it can be said a CH-group is in this case involved. Beside the aromatic carbon atom, there is no other signal in the ^{13}C-spectrum. In conclusion, aliphatic or other carbon atoms such as carbonyl- or carbon acid groups are not present.
14.0 (s, 1H) ppm: In the ^{1}H-spectrum, a strong downfield shift and broad peak signal is particularly noticeable. The broadened signal is evidence that this should be an amine or hydroxyl-proton, since the broadened signal is resulting from the ability to form hydrogen bonds. Literature research utilizing the SDBS database reveals such downfield shift can be triggered by pyrazole derivatives. Since the afore deduced aromatic C-atom corresponds to this assumption, pyrazole will be assumed as the skeletal structure for the unknown structure (Figure 7).

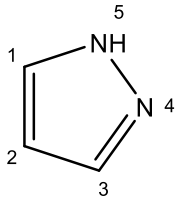

Figure 7: Skeletal pyrazole-structure as assumed for substance 3.

8.29 (s, 2H): Additionally, the spectrum presents a singlet, which can be assigned to two equivalent H-atoms. The downfield shift of the signal indicates these hydrogens are to be found on aromatic atoms. Since these protons are magnetically equivalent, they must be symmetrically arranged in the molecule. This is the case for H-atoms which are found at position 1-C and 3-C. The symmetry results from mesomerism in the molecule. This arrangement concurs with the signal from the ^{13}C-spectrum on which at least a CH-group is shown. Due to symmetry of the molecule, the carbon atoms at positions 1 and 3 form a single signal in the ^{13}C-NMR-spectrum. Since no other signal appears on the ^{13}C-spectrum and the carbon atom at position 2 is not

equivalent to carbon at position 1 and 3, a heteroatom must be present. Taking the binding relationships into consideration, a nitrogen atom probable.

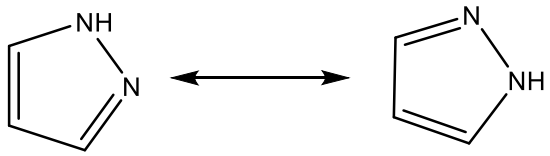

Figure 8: Mesomerism in pyrazole.

In conclusion, the spectroscopic characterisation suggests a Triazole as the unknown compound. Comparison with the literature data reveals the spectrum of the unknown substance perfectly matches data of 1,2,4-triazole reported in literature.
The signals are assigned as following (Figure 9).

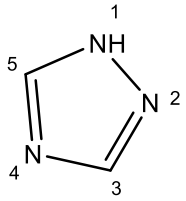

Figure 9:Numbered structure of 1,2,4-triazole for signal assignment.

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^{1}H-NMR (400 MHz, (DMSO-*d*6)): δ = 14.04 (s, 1H, N-H), 8.29 (s, 2H, 3-H, 5-H) ppm.
^{13}C-NMR (100 MHz, (DMSO-*d*6)): δ = 146.8 (+, 3-C, 5-C) ppm.

Reference:

[1] Gregory R. Fulmer, Alexander J. M. Miller, Nathaniel H. Sherden, Hugo E. Gottlieb, Abraham Nudelman, Brian M. Stoltz, John E. Bercaw, Karen I. Goldberg, Organometallics, 2010, 29, 2176-2179.

[2] Spectral Database for Organic Compounds, SDBS, National Institute of Advanced Industrial Science and Technology (AIST), Japan. (zuletzt aufgerufen: 27.09.2021) https://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre_index.cgi.

[3] J. Namyslo, Skript: Strukturaufklärung organischer Verbindungen, Institut für Organische Chemie, TU Clausthal: Clausthal-Zellerfeld, 2020.

Note: in this template report, only SDBS[2] is cited as a source for the literature spectra. However, on your turn-in, please cite the particular paper you received the spectroscopic information from.