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Asborin: The Carbaborane Analogue of Aspirin

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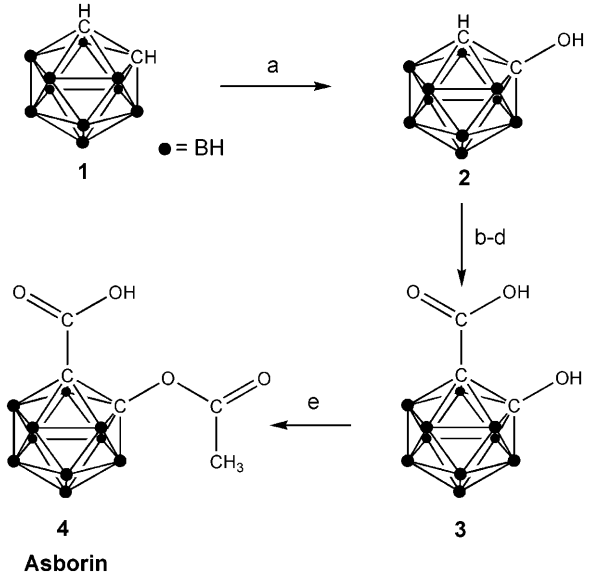
Aspirin, the acetyl ester of salicylic acid, was first discovered in the late 1800s and became one of the most world-renowned drugs. Its fame is rooted in its ability to treat a wide range of symptoms such as fever, inflammation, and pain. Aspirin represents an advancement of the previously applied salicylic acid, which was obtained from myrtle leaf decoction or willow bark extract, but was found to be less active and to cause serious gastrointestinal irritation.[1] Later on, other chemical modifications of aspirin were investigated with the aim of improving its pharmacological profile. The influence of additional substituents on the phenyl ring and modification of the acetoxy and carboxy groups were studied,[2] and even the phenyl core of aspirin was replaced by the organometallic ferrocene moiety.[3] However, none of these derivatives could substitute for aspirin. We have now modified aspirin by replacing the phenyl ring with a three-dimensional and highly hydrophobic carbaborane cluster.

Carbaboranes are already known in medicinal chemistry as phenyl group mimetics, and analogues of tamoxifen,[4] trimethoprim,[5] and transthyretin amyloidosis inhibitors derived from flufenamic acid and diflunisal,[6] two nonsteroidal anti-inflammatory drugs (NSAIDs), have been presented.

The focal point of our research was a high-yield synthesis of asborin, the carbaborane analogue of aspirin, and the study of its pharmacological behavior. Asborin is obtained in three steps (Scheme 1) starting from *ortho*-dicarba-*closo*-dodecaborane(12) (**1**). In the first step one of the carbaborane carbon atoms is hydroxylated according to a protocol described by Endo and co-workers.[7] The monolithiated carbaborane is treated with trimethyl borate to give a carbaboranyl boronic ester in situ. The ester is then oxidized with peracetic acid. The second step is carboxylation of the second cluster carbon atom. Both the OH proton and the remaining carbaborane CH proton of **2** can be removed with *n*-butyllithium, and reaction of the resulting dilithium salt with gaseous carbon dioxide gives 1-hydroxy-1,2-dicarba-*closo*-dodecaborane(12)-2-carboxylic acid (**3**) in greater than 99% yield after acidic workup. Compound **3**, with an *ortho* arrangement of hydroxy and carboxy groups, is the carbaborane analogue of salicylic acid. Finally, 1-acetoxy-1,2-dicarba-*closo*-dodecaborane(12)-2-carboxylic acid (asborin, **4**) is obtained quantitatively simply by stirring **3** in acetyl chloride. The use of acetyl chloride rather than acetic anhydride is advantageous, as it can easily be removed under reduced pressure due to its lower boiling point of 52 °C. Crystals of **4** suitable for X-ray crystallography (Figure 1) were obtained from chloroform; hydrogen-bonded dimers are formed via the carboxy groups.



Scheme 1. Synthesis of asborin (**4**): a) according to reference [7]; b) *n*BuLi, Et_2O, 0 °C→RT; c) CO_2, RT; d) HCl, RT, >99%; e) CH_3COCl, RT, >99%.

Asborin, with its hydrophobic cluster framework and hydrophilic carboxy group, is remarkably amphiphilic: It dissolves in water and nonpolar organic solvents.

Aspirin is one of the smallest members of the NSAID family. These compounds are structurally very diverse, but all of them are capable of inhibiting cyclooxygenase (COX). These enzymes

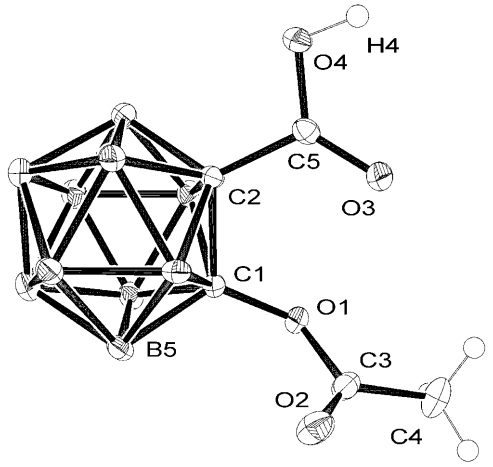


Figure 1. ORTEP of asborin (**4**) with BH protons omitted for clarity; thermal ellipsoids are drawn at 50% probability.

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catalyze the conversion of arachidonic acid to prostaglandin H_2 (PGH_2), the key compound in the prostanoid system. COX itself is present in different isoforms, of which COX-1 and COX-2 are the best scrutinized.[8] The pharmacological action of aspirin is unique among the NSAIDs, as it modifies the enzymes covalently by acetylating an active site serine residue.[1,9] When the serine residue is acetylated, PGH_2 cannot be formed, and the synthesis of all further prostaglandins, prostacyclins, and thromboxanes is blocked.

COX inhibition can generally be studied by using different assay systems. Free enzyme preparations are normally applied first to identify the presence of any inhibition. Analyses using cell systems or whole-blood samples are then used to test the compounds in a more complex environment. Animal models in vitro finalize the preclinical testing.

In our case, preliminary investigations at a high inhibitor concentration were initially carried out. An enzyme-linked immunosorbent assay (ELISA)-based COX inhibitor screen with the free enzymes of both COX-1 and COX-2 was used to determine whether asborin shows COX inhibition and isoform selectivity. At an asborin concentration of 500 μM, COX-1 is about 50% inhibited; COX-2 activity is also decreased by the lesser extent of 28%. Therefore, asborin is, like aspirin, capable of inhibiting COX enzymes. By showing higher inhibition potency toward COX-1, asborin also reveals the same trend in isoform selectivity as aspirin, which is, in both cases, not very pronounced.[10] With respect to the free enzyme system, asborin is slightly less active than aspirin, but the behavior in a whole-blood or cell-based assay is expected to be different. Because the enzyme entrance in living systems is buried in the membrane interior,[11] the more hydrophobic cluster compound may be advantageous. Complete IC_{50} determinations and studies on the mechanism of COX inhibition as well as biodistribution and biodegradation are underway.

Experimental Section

All reactions were carried out under N_2 atmosphere by using standard Schlenk techniques. The solvents were purified by an SPS-800 Series solvent purification system. Compound **1** was donated by INEOS (A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Science, Moscow, Russian Federation). Compound **2** was synthesized according to reference [7]. Trimethylborate and acetyl chloride were distilled under N_2 before use. All other chemicals were used as purchased. IR spectra were recorded on a PerkinElmer System 2000 FTIR spectrometer using a KBr disk. ^{1}H, ^{13}C, and ^{11}B NMR spectra were recorded on an AVANCE DRX 400 spectrometer (Bruker). Chemical shifts for the ^{1}H, ^{13}C, and ^{11}B NMR spectra are reported in ppm at 400.13, 100.63, and 128.38 MHz, respectively, with tetramethylsilane as standard for the first two and $BF_3(OEt_2)$ as external standard for the latter. MS data were recorded on an FTICR MS Bruker-Daltonics ESI mass spectrometer (APEX II, 7 T). Elemental analyses were carried out on a Vario EL instrument (Heraeus). Melting points were determined in capillaries (Gallenkamp). Crystals for molecular structure determination were grown at room temperature. Crystallographic data for **4** were collected on a CCD Oxford Xcalibur S diffractometer ($\lambda(Mo_{K\alpha})=0.71073$ Å) in ω and ϕ scan mode. Semi-empirical from equivalents absorption corrections were carried out with SCALE3 ABSPACK and the structure was solved with direct methods.[12,13] Structure refinement was carried out with SHELXL-97.[14] All non-hydrogen atoms were refined anisotropically, all H atoms were located on difference Fourier maps and refined freely. A commercially available COX inhibitor screening assay from Cayman Chemical (Catalog No. 560131) was applied for preliminary activity and selectivity investigations. The absorption at 415 nm was measured using a Flashscan Analytik S12 (Jena AG).

3: 1-Hydroxy-1,2-dicarba-*closo*-dodecaborane(12) (0.73 g, 4.56 mmol) was dissolved in Et_2O (50 mL) and treated with *n*BuLi in *n*-hexane (3.6 mL, 2.7 M, 9.71 mmol, 2.13 equiv) at 0 °C. The mixture was stirred for 1 h at 0 °C and an additional 30 min at room temperature. Dry CO_2 was then bubbled through the reaction mixture for 1 h at room temperature. Evaporation of the solvent in vacuo gave a white solid. Aqueous HCl (30 mL, 1 M) was added to the solid. The suspension was then extracted with Et_2O (4×30 mL). The combined ethereal layers were dried over $MgSO_4$ and concentrated in vacuo to yield a white crystalline solid. Yield of **3**: 0.93 g (> 99%); elemental analysis calcd (%) for $C_3B_{10}H_{12}O_3$: C 17.64, H 5.92; found: C 17.36, H 5.77; mp: 205–208 °C; ESIMS(−) (CH_3OH): *m/z* (%): 202.9 (100) $[M-H]^-$, 159.0 (31) $[M-COOH]^-$; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta=3.14$–1.20 ppm (vbr m, 10 H, $C_2B_{10}H_{10}$) (the value for the acidic protons is concentration dependent); ^{11}B NMR (128 MHz, $CDCl_3$, 25 °C, TMS): $\delta=-3.0$ (d, $^1J_{(B,H)}=141$ Hz, 1 B, $C_2B_{10}H_{10}$), −9.8 (d, $^1J_{(B,H)}=141$ Hz, 2 B, $C_2B_{10}H_{10}$), −11.3 (d, $^1J_{(B,H)}=154$ Hz, 3 B, $C_2B_{10}H_{10}$), −12.4 ppm (d, $^1J_{(B,H)}=141$ Hz, 4 B, $C_2B_{10}H_{10}$); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta=165.8$ (s, COOH), 101.2 (s, $C_{cluster}O$), 70.0 (s, $C_{cluster}$-COOH); IR (KBr): $\tilde{\nu}=3416$ (m, ν(O–H)), 2964 (m), 2590 (s, ν(B–H)), 1724 (s, ν(COO)), 1702 (s, ν(COO)), 1431 (m), 1416 (m), 1383 (m), 1268 (s, $\nu(C_{cluster}-O)$), 1136 (w), 1098 (w), 1018 (m), 948 (w), 861 (w), 815 (w), 737 (w), 721 (m), 662 (w), 611 (w), 583 (w) 522 (w) 462 cm^{-1} (w).

4: 1-Hydroxy-1,2-dicarba-*closo*-dodecaborane(12)-2-carboxylic acid (0.28 g, 1.37 mmol) was dissolved in acetyl chloride (10 mL) and stirred for 3 h at room temperature. The conversion is quantitative. **4** is obtained as a white solid after removal of the solvent. Yield: 0.34 g (> 99%); elemental analysis calcd for $C_5B_{10}H_{14}O_4$: C 24.38, H 5.73; found: C 24.68, H 5.68%; mp: 146 °C; ESIMS(−) (CH_3OH/$CHCl_3$): *m/z*: 492.1 (54) $[2M-H]^-$, 245.0 (52) $[M-H]^-$, 236.1 (25) $[2M-B]^-$, 201.0 (42) $[M-COOH]^-$, 159.0 (100) $[M-COO-CH_3CO]^-$, (different fragments are observed when using different solvents); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta=3.68$–1.48 (vbr m, 10 H, $C_2B_{10}H_{10}$), 2.11 (s, 3 H, CH_3) (the value for the acidic proton is concentration dependent); ^{11}B NMR (128 MHz, $CDCl_3$, 25 °C, TMS): $\delta=-3.0$ (d, $^1J_{(B,H)}=154$ Hz, 1 B, $C_2B_{10}H_{10}$), −7.9 (d, $^1J_{(B,H)}=154$ Hz, 1 B, $C_2B_{10}H_{10}$), −11.4 (d, 4 B, $C_2B_{10}H_{10}$), −12.0 ppm (d, 4 B, $C_2B_{10}H_{10}$, $^1J_{(B,H)}$ could not be resolved for the last signals, and due to the broad signals the number of boron atoms could not be determined unambiguously); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, TMS): $\delta=165.1$ (s, COOH), 161.5 (s, COOR), 95.1 (s, $C_{cluster}O$), 73.3 (s, $C_{cluster}$-COOH), 21.0 ppm (t, $^1J_{(C,H)}=131$ Hz, CH_3); IR (KBr): $\tilde{\nu}=3439$ (w, ν(O–H)), 2987 (m), 2851 (m), 2638–2565 (s, ν(B–H)), 1805 (s, ν(C=O)), 1736 (s, ν(COOH)), 1420 (s), 1368 (s), 1281 (s), 1212 (w), 1159 (s), 1032 (m), 1019 (s), 940 (w), 925 (w), 888 (w), 860 (m), 849 (w), 793 (w), 721 (w), 698 (w), 670 (w), 648 (w), 613 (w), 522 (w), 470 cm^{-1} (w).

Structural data for **4** obtained from $CHCl_3$: $C_5H_{14}B_{10}O_4$, $M_r=246.26$, triclinic, space group $P\bar{1}$, $a=690.6(3)$, $b=968.1(4)$, $c=1029.4(4)$ pm, $\alpha=76.62(3)$, $\beta=74.94(4)$, $\gamma=74.05(4)°$, $V=0.6294(4)$ nm^3, $Z=2$, $\rho_{calcd}=1.299$ Mg m^{-3}, $\mu=0.083$ mm^{-1}, $2.77<\theta<30.50°$, $R=0.0574$, $wR=0.1593$. The measured crystal revealed a minor twin component of 18%, which was, however, not included in the structure solution and refinement.

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CCDC 08994 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

The COX inhibition of asborin was tested at a final concentration of 500 μM twice and in duplicates according to the instruction manual. COX-1 and COX-2 were inhibited by 49±5% and 28±11%, respectively.

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Keywords: aspirin analogues · carbaboranes · cluster compounds · drug design · inhibitors

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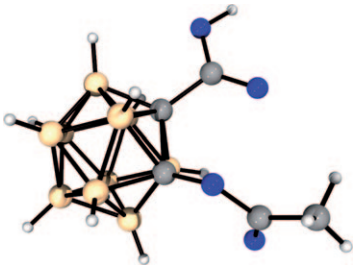

Asborin, the carbaborane analogue of aspirin, was obtained by a high-yield synthetic procedure and proved to be an active cyclooxygenase (COX) inhibitor (H: white, B: beige, C: gray, O: blue).

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