

Supplementary Information

Isolation, expression and biochemical characterization of recombinant hyoscyamine-6 β -hydroxylase from *Brugmansia sanguinea* – tuning the scopolamine production

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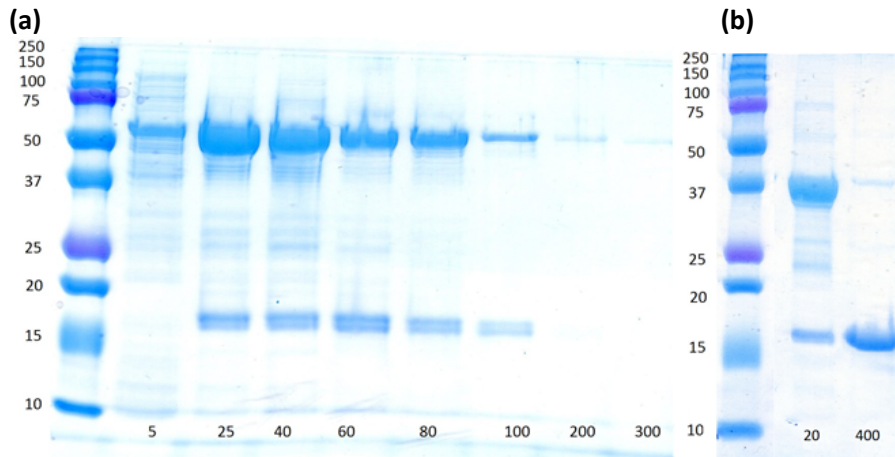


Figure S1. (a) Purification of SUMO-BsH6H analyzed by coomassie blue stained SDS-page. Amounts of imidazole used to elute the protein are indicated under each line (mM). (b) After cleavage of the SUMO-tag, the native BsH6H remains in the flowthrough (first lane).

		Fe-binding	
<i>Hyoscyamus niger</i>	MATFVSNWSTKSVSESFIAPIQKRAEKDVPIGNDVPIIDLQQHHLIVQQITKACQDFGL	60	
<i>Hyoscyamus senecionis</i>	MATFVSNWSTKSVTESFIAPIQKRAEKDVPIGNVPIIDLQQHHLIVQQITKACQDFGL	60	
<i>Datura stramonium</i>	MATFVSNWSTNNVSESFIAPILEKRAEKDVPLGNDVPIIDLQQDHLIVQQITKACQDFGL	60	
<i>Datura metel</i>	MATLVSNWSTNNVSESFIAPILEKRAEKDVPLGNDVPIIDLQQDHLIVQQITKACQDFGL	60	
<i>Duboisia myoporoides</i>	-----DVPIIDLKQDHIIVVQQINKACQDFGL	27	
<i>Brugmansia sanguinea</i>	MATFVSNWSTNNVSESFIAPILEKRAEKDVPLGNDVPIIDLQQDHLIVQQITEACQDFGL	60	
<i>Brugmansia arborea</i>	MATFVSNWSTNNVSESFIAPILEKRAEKDVPLGNDVPIIDLQQDHLIVQQITKACQDFGL	60	
<i>Atropa baetica</i>	MATFVSNWSTNNVSESFVAPLEKRAEKDVPLGNDVPTIDLQQDHLVVVQQITKACQDFGL	60	
<i>Atropa belladonna</i>	MATLVSNWSTNNVSESFVAPLEKRAEKDVPLGNDVPIIDLQQDHLVVVQQITKACQDFGL	60	
<i>Scopolia parviflora</i>	MATLVSNWSSNNVSESFIAPILEKRAEKDVPLGNDVPMIDLQQEHHLVVQQITKACQDFGL	60	
<i>Anisodus acutangulus</i>	MATLVSNWSTNNVSESFKAPLEKRAEKDVPLGNDVPIIDLQQDHLVVVQQITKACQDFGL	60	
<i>Anisodus tanguticus</i>	MATLVSNWSSNNVSESFIAPLDKRAEKVPLGNDVPIIDLQQDHHFVVQQITKACKDFGL	60	
<i>Anisodus luridus</i>	MATFVSNWSSNNVSESFIAPLDKRAEKVPLGNDVPIIDLQQDHHFVVQQITKACKDFGL	60	
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	Fe-binding		
<i>Hyoscyamus niger</i>	FQVINHGFPEELMLETMEVCKEFFALPAEEKEKFKPKGEAAKFELPLEQKAKLYVEGEQL	120	
<i>Hyoscyamus senecionis</i>	FQVINHGFPEELMLETMEVCKEFFALPAEEKEKFKPKGEAAKFELPLEQKAKLYIEGEQL	120	
<i>Datura stramonium</i>	FQVINHGVPEKLMVEAMEVYKEFFALPAEEKEKFQPKGEPAKFELPLEQKAKLYVEGERR	120	
<i>Datura metel</i>	FQVINHGVPEKLMVEAMEVYKEFFALPAEEKEKFQPKGEPAKFELPLEQKAKLYVEGERR	120	
<i>Duboisia myoporoides</i>	FQVINHGFPEKLMETMDVCKEFAFALPAEEKEKLQPKGEPAKFELPLEQKAKLYVEGEQL	87	
<i>Brugmansia sanguinea</i>	FQVINHGFSEKLMAETMEVCKEFFALPAEEKEKLQPKGEPAKFELPLEQKAKLYVEGEQL	120	
<i>Brugmansia arborea</i>	FQVINHGFSEKLMAETMEACKEVFALPAEEKEKLQPKGEPAKFELPLEQKAKLYVEGEQL	120	
<i>Atropa baetica</i>	FQVINHGLPEKLMAETMDVCKEFAFALPAEEKEKLQPKGEPAKFELPLEQKAKLYVEGEQL	120	
<i>Atropa belladonna</i>	FQVINHGLPEKLMAETMDVCKEFAFALPAEEKEKLQPKGEPAKFELPLEQKAKLYVEGEQL	120	
<i>Scopolia parviflora</i>	FQVINHGFPEKLMAETMKVCKEFAFALPAEEKEKLQPKGEPAKFELPLEQKAKLYIEGEQL	120	
<i>Anisodus acutangulus</i>	FQVINHGFPEKLMAETMKVCKEFAFALPAEEKEKLQPKGKPAKFELPLEQKAKLYIEGEQL	120	
<i>Anisodus tanguticus</i>	FQVINHGFPEKLMEVETMEVCKEFFALPAEENEKLQPKGKPAKFELPLEQKAKLYIEGEQL	120	
<i>Anisodus luridus</i>	FQVINHGFPEKLMAETMKLCKEFAFALPAEEKEKLQPKGKPAKFELPLEQKAKLYIEGEQL	120	
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Hyoscyamus niger SNEEFlyWKDTLAHGCHPLDQDLVNSWPEKPAKYREVVAKYSVEVRKLTMRMLDYICEGL 180
Hyoscyamus senecionis SNEEFlyWKDTLAHGCHPLDQDLVNSWPEKPAKYREVVAKYSVEVRKLTMRMLDYI~~SE~~GL 180
Datura stramonium CNEEFlyWKDTLAHGCHPLHEEL~~LN~~SWPEKPTTYR~~DI~~AKYSVEVRKLTMRILDYICEGL 180
Datura metel CNEEFlyWKDTLAHGCHPLHEEL~~LN~~SWPEKPTTYR~~DI~~AKYSVEVRKLTMRILDYICEGL 180
Duboisia myoporoides SNDEFYyWKDTLAHGCHPLHE~~DL~~VNSWPKPSTYRE~~LMS~~KYSVEVRKLTMRILDYICEGL 147
Brugmansia sanguinea SNEEFlyWKDTLAHGCHPLDEEL~~LN~~SWPEKPTTYR~~DM~~AKYSVEVRKLTMRILDYICEGL 180
Brugmansia arborea SNDEFlyWKDTLAHGCHPLDEEL~~LN~~SWPEKPTTYR~~DM~~AKYSVEVRKLTMRILDYICEGL 180
Atropa baetica SDEAFlyWKDTLAHGCHPLDEELVNSWPEKPATYREVVAKYSVEVRKLTMRILDYICEGL 180
Atropa belladonna SDEAFlyWKNTLAHGCHPLDEELVNSWPEKPATYREVVAKYSVEVRKLTMRILDYICEGL 180
Scopolia parviflora SNEEFYyWKDTLAHGCHPLDE~~KL~~VNSWPEKPATYREVVAKYSVEVRKLTMRMLDYICEGL 180
Anisodus acutangulus SNGELFyWKDTLAHGCHPLDEELVNSWPEKPATYREVV~~SK~~YSVEVRKLTMRMLDYICEGL 180
Anisodus tanguticus SNEEFYyWKDTLAHGCHPLDEEL~~IN~~SWPEKPATYREVA~~AK~~YSVE~~ARK~~LTMRLDYICEGL 180
Anisodus luridus SNDEFYyWKDTLAHGCHPLDEELVNSWPEKPTTYREVA~~AK~~YSVE~~ARK~~LTMRLDYICEGL 180
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2-ketoglutarate binding
Hyoscyamus niger GLKLGyFDNELSQIQMMLTNYPPCPDPSSTLGSGGHYDGNLITLLQQDL~~PGL~~QQLIVKD 240
Hyoscyamus senecionis GLKLGyFDNELSQIQMMLTNYPPCPDP~~ST~~TLGSGGHYDGNLITLLQQDL~~PGL~~QQLIVKD 240
Datura stramonium GLKLGyFDNELTQIQML~~LAN~~YPPSCDP~~PS~~STIGSGGHYDGNLITLLQQDL~~VGL~~QQLIVKD 240
Datura metel GLKLGyFDNELTQIQML~~L~~AN~~Y~~PPSCDP~~PS~~STIGSGGHYDGNLITLLQQDL~~VGL~~QQLIVKD 240
Duboisia myoporoides GLKLGyFDNELCQIQML~~L~~TNYPPCPDPSSTLGSGGHYDGNLITLLQQDL~~PGL~~QQLIVKD 207
Brugmansia sanguinea GLKLGyFDNELSQIQML~~L~~TNYPPCPDPSSTLGSGGHYDGNLITLLQQDL~~PGL~~QQLIVKD 240
Brugmansia arborea GLKLGyFDNELSQIQML~~L~~TNYPPCPDPSSTLGSGGHYDGNLITLLQQDL~~PGL~~QQLIVKD 240
Atropa baetica GLKLGyFDNELSQIQMMLTNYPPCPDPSSTLGSGGHYDGNLITLLQQ~~NLPGL~~QQLIV~~ED~~ 240
Atropa belladonna GLKLGyFDNELSQIQMMLTNYPPCPDPSSTLGSGGHYDGNLITLLQQ~~NLPGL~~QQL-~~IED~~ 239
Scopolia parviflora ELKLGyFDNELSQIQMMLTNYPPCPDPSSTLGSGGHYDGNLITLLQQDL~~PGL~~QQLIVKD 240
Anisodus acutangulus GLKLGyFDNELSQIQMML~~AN~~YPPCPDPSSTLGSG~~AH~~YDGN~~VIT~~LLQQDL~~PGL~~QQLIVKD 240
Anisodus tanguticus GLKLGyFDNELSQIQMMLTNYPPCPDPSSTLGSGGHYDGNLITLLQQDL~~PGL~~QQLIVKD 240
Anisodus luridus GLKLG~~H~~FDNELSQIQMMLTNYPPCPDPSSTLGSGGHYDGNLITLLQQDL~~PGL~~QQLIVKD 240
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Fe-binding
Hyoscyamus niger ATWIAVQPIPTAFVVLNGLTLKVI~~TNE~~KFE~~GS~~IHRV~~TD~~PTRDRVSIATLIGPDYSCTIE 300
Hyoscyamus senecionis STWIAVQPIPTAFVVLNGLTLKVI~~SNE~~KFE~~GS~~IHRV~~TD~~PTRDRVSIATLIGPDYSCTIE 300
Datura stramonium DKWIAVEPIPTAFVVLNGLTLK~~VMS~~NEKFE~~GS~~IHRV~~TH~~PTRNR~~ISIG~~TLIGPDYSCTIE 300
Datura metel DKWIAVEPIPTAFVVLNGLTLK~~VMS~~NEKFE~~GS~~IHRV~~TH~~PIRNR~~ISIG~~TLIGPDYSCTIE 300
Duboisia myoporoides GNWIAVEPIPTAFVVLNGLTLKVI~~TNE~~KLE~~GS~~IHRV~~TN~~PTRDRVSIATLIGPDYSCTIE 267
Brugmansia sanguinea ANWIAVEPIPTAFVVLNGLTLKVI~~SNE~~KFE~~GS~~IHRV~~TN~~PT~~S~~DRVSIATLIGPDYSCTIE 300
Brugmansia arborea ANWIAVEPIPTAFVVLNGLTLKVI~~SNE~~KFE~~GS~~IHRV~~TN~~PTRDRVSIATLIGPDYSCTIE 300
Atropa baetica AKWIAVEPIPTAFVVLNGLTLKVI~~TNE~~KFE~~GS~~IHRV~~TN~~PTRDRVSIATLIGPDYSCTIE 300
Atropa belladonna AKWIAVEPIPTAFVVLNGLTLKVI~~TNE~~KFE~~GS~~IHRV~~TN~~PTRDRVSIATLIGPDYSCTIE 299
Scopolia parviflora GNWIAVE~~PN~~PTAFVINLGLTLKVI~~TNE~~KFE~~GS~~IHRV~~AD~~PTRDRVSIATLIGPDYSCTIE 300
Anisodus acutangulus DNWIAVEPIPTAFVVLNGLTLKVI~~TNE~~KFE~~GS~~IHRV~~TN~~PTRDRVSIATLIGPDYSCTIE 300
Anisodus tanguticus DNWIAVEPIPTAFV~~IN~~LGLTLKVI~~TNE~~KFE~~GS~~IHRV~~TD~~PTRDRVSIATLIGPDYSCTIE 300
Anisodus luridus DNWIAVEPIPTAFV~~IN~~LGLTLKVI~~TNE~~KFE~~GS~~IHRV~~TN~~PTRDRV~~T~~IATLIGPDYSCTIE 300
.***:*** *****:*****:*****:** *****:** *****:** *****:** *****:**
Hyoscyamus niger PAKELLNQDNPPLYKPY~~SY~~SEFADIYLSDKSDYDSGVKPYKIN~~V~~ 344
Hyoscyamus senecionis PAKELLNQDNPPLYKPY~~SY~~AEFA~~DI~~YLSDKSDYDAGVRPYKIN~~V~~ 344
Datura stramonium PIKELLSQENPPLYKPY~~PA~~AKFAEYLSDKSDYDAGVKPYKIN~~-~~ 343
Datura metel PIKELISQENPPLYKPY~~PA~~AEFAEYLSDKSDYDAGVKPYKIN~~-~~ 343
Duboisia myoporoides PAKELLSQDNPPLYKPY~~SY~~AEFAEYLSDKS~~GY~~DAGVKPYKLN~~-~~ 310
Brugmansia sanguinea PAKELVNQENPPLYKPY~~PA~~AEFAEYLSDKSD~~F~~DAGVKPYKIN~~V~~ 344
Brugmansia arborea PAKELVSQENPPLYKPY~~VE~~FAEYLSDKSDYDA~~AV~~KPYKIN~~-~~ 343
Atropa baetica PAKELLSQDNPPLYKPY~~SY~~AEFAEYLSDKSDYDAGVKPYKIN~~V~~ 344
Atropa belladonna PAKELLSQDNPPLYKPY~~SY~~AEFAEYLSDKSDYDAGVKPYKINA~~A~~ 343
Scopolia parviflora PGKELLSQDNPPLYKPY~~TE~~FAEYLSDKS~~GY~~----- 333
Anisodus acutangulus PAKELLSQDNPPLYKPY~~PA~~AEFAEYLSDKS~~GY~~DAGVKPYKINA~~A~~ 344
Anisodus tanguticus PAKELLSQDNPPLYKPY~~PA~~AEFAEYLSDKS~~GY~~DAGVKPYKINA~~A~~ 344
Anisodus luridus PAKELLSQDNPPLYKPY~~PA~~AEFAEYLSDKS~~GY~~DAGVKPYKINA~~A~~ 344
* ***:*** *****:*****:*****:** *****:** *****:** *****:** *****:**

Figure S2. Multiple sequence alignments for comparison of BsH6H with other reported H6H homologues. Two iron binding and one co-factor region are boxed. Red letters indicate different residues with regard to the consensus. Blue highlighted letters depict significantly different residues in *Brugmansia sanguinea*-H6H compared to other H6H homologues. Accession numbers: ABG89397.1 (*H. niger*); AFP99876.1 (*H. senecionis*); ALD59774.1 (*D. stramonium*); AAQ04302.1 (*D. metel*);

AQU12714.1 (*D. myoporoides*); ALD59773.1 (*B. arborea*); ABR15749.1 (*A. baetica*); AEM91979.1 (*A. belladonna*); AAY53932.1 (*S. parviflora*); ABM74185.1 (*A. acutangulus*); AAQ75700.1 (*A. tanguticus*); AGL76991.1 (*A. luridus*).

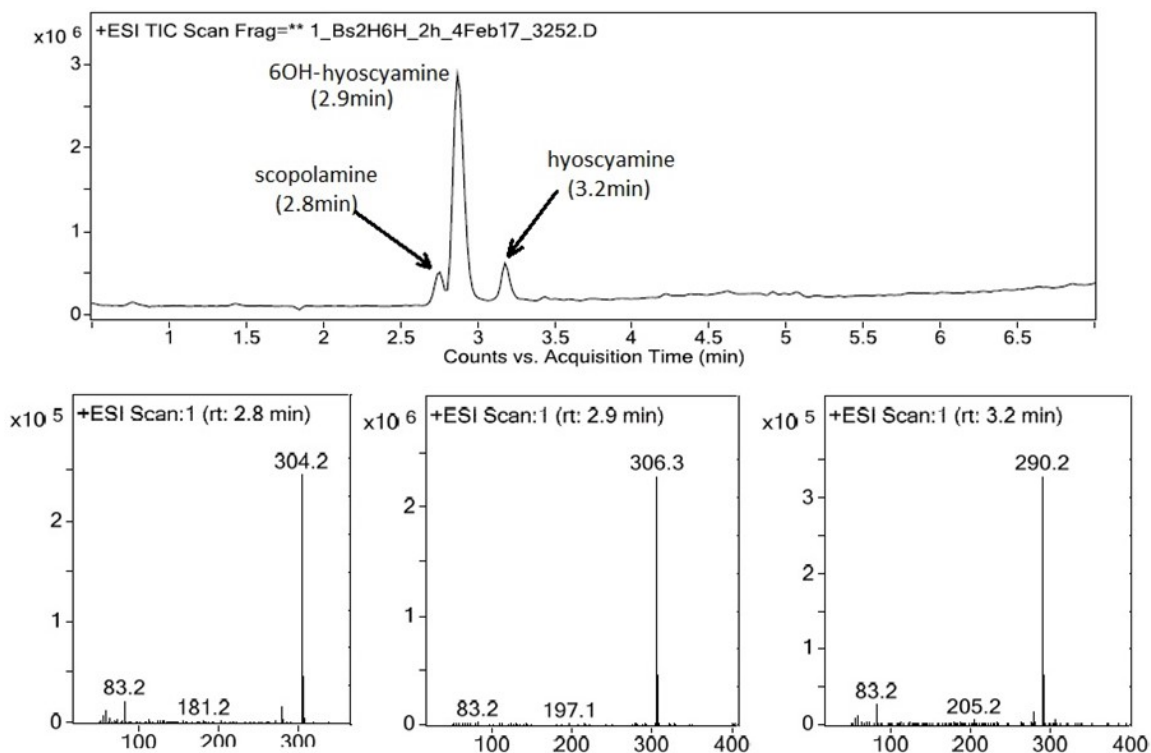


Figure S3. LCMS analysis of the transformation of hyoscyamine **1** (3.2 min) to intermediate **2** (2.9 min) and scopolamine **3** (2.8 min) by BsH6H and MS identification of products.