

A comprehensive NMR analysis of hyperforin (**1**) as well as for 2-acetyl hyperforin (**2**) was performed using two-dimensional ^1H homocorrelation (DQF-COSY, NOESY) and $^1\text{H}^{13}\text{C}$ heterocorrelation experiments (HMQC, HMBC). In the ^1H NMR spectrum of **2**, 30 signals (Table S1) belonging to heterotopic H-atoms could be assigned by correlation *via* scalar couplings in COSY experiments (22 detected correlations) and dipolar couplings in NOESY experiments (28 detected correlations) (Table S2). The (E)-methyl groups in the three dimethyl allyl and in the dimethyl butenyl moiety could be assigned by strong NOE interactions with the protons at the neighbouring olefinic carbons atoms (Fig. S1). The ^{13}C NMR resonances could be assigned by 30 $^1\text{H}^{13}\text{C}$ correlations *via* one bond detected in the HMQC experiment (i.e., by magnetization transfer *via* $^1\text{J}_{\text{CH}}$) and by 101 $^1\text{H}^{13}\text{C}$ correlations *via* two and three bonds observed in HMBC experiments (i.e., by magnetization transfer *via* $^2\text{J}_{\text{CH}}$ and $^3\text{J}_{\text{CH}}$). Typically, three $^1\text{H}^{13}\text{C}$ correlations were detected for a specific C-atom, thus providing redundant information for the assignments (Table S2). For example, the ^{13}C NMR resonances for all olefinic carbon atoms in **1** and **2** showed long range CH-correlations *via* $^2\text{J}_{\text{CH}}$ and $^3\text{J}_{\text{CH}}$ with ^1H signals of the attached methyl groups (Fig. S2), as well as with ^1H signals of the neighbouring methylene groups 16, 21, 26 and 31, respectively (Table S2). Regiospecific assignments for the side chains were obtained by long range correlations of ^{13}C signals for carbon atoms located in the cyclic moiety of hyperforin with ^1H signals of protons located in the side chains. For example, C-2 of **2** showed long range correlations to H-8, H-26 and H-31, whereas C-3 and C-4 correlated exclusively with H-26 (Table S2). These data confirm the correctness of the structure indicated in **2** for the O-acetyl derivative of hyperforin.

Additional information was obtained from one-dimensional ^{13}C NMR spectra and two-dimensional INADEQUATE experiments of the multiply ^{13}C labeled hyperforin sample from the experiment with $[\text{U-}^{13}\text{C}_6]\text{glucose}$ (Table S2, Fig. 5). Eleven pairs of ^{13}C NMR signals were detected at specific double quantum frequencies connecting signals of aliphatic and

olefinic carbon atoms by magnetization transfer via one bond $^{13}\text{C}^{13}\text{C}$ couplings (i.e., via $^1J_{\text{CC}}$).

On this basis, the assignments for 22 carbon signals could be double checked.

Table S1. NMR data of hyperforin (1) and 2-acetyl hyperforin (2)

Position	Chemical Shift				Coupling Constants ^b	
	¹³ C chemical shift (δ, ppm)		¹ H chemical shift (δ, ppm)		J _{HH} (Hz)	J _{CC} (Hz)
	1	2	1	2	2	2
1	60 broad	58.70				37.0 (9), 47.6 (2)
2	n.d.	164.47				79.0 (3), 47.6 (1)
3	122.12	135.28				53.1 (4), 79.0 (2)
4	n.d.	195.04				53.1 (3), 37.0 (5)
5	82 broad	85.80				37.0 (4, 9)
6	49.10	51.08				36.1 (14)
7	42.95	42.82	1.64 (m)	1.82 (m)		35.0 (8)
8	40.82	39.42	Ha: 1.30 (m) Hb: 1.82 (m)	Ha: 1.40 (m), Hb: 2.02 (m)		35.0 (7)
9	208.85	207.60				37.0 (1,5)
10	211.78	210.03				
11	43.00	44.08	2.02 (m)	1.89 (m)		34.1 (12)
12	21.16	20.75	0.94 (d)	1.04 (d)	6.5	34.1 (11)
13	21.98	21.61	0.99 (d)	0.95 (d)	6.6	
14	15.31	14.64	0.88 (s)	0.96 (s)		36.1 (6)
15	37.92	37.67	1.59 (m)	Ha: 1.50 (m), Hb: 1.70 (m)		
16	25.42	25.66	Ha: 1.85 ^a	Ha: 1.99 ^a , Hb: 1.81 ^a		43.3 (17), 4.2 (20)
17	126.04	125.67	4.92 (m)	4.96 (m)		43.3 (16), 3.5 (20)
18	131.84	132.08				42.2 (19)
19	18.09	17.95	1.49 (s)	1.58 (s)		42.2 (18)
20	25.87	25.86	1.56	1.63		
21	28.62	28.17	Ha: 1.95 (m) Hb: 1.65 (m)	Ha: 2.05 ^a Hb: 1.74 ^a	7.2, 14.9	44.0 (22), 4.2 (25)
22	123.77	123.57	4.90 (m)	4.99 (m)		43.9 (21), 3.8 (25)
23	134.25	133.85				42.2 (24)
24	18.15	18.02	1.48 (s)	1.53 (s)		42.2 (23)
25	25.97	25.89	1.59	1.65		
26	22.50	24.72	Ha: 3.05 (dd) Hb: 2.99 (dd)	Ha: 2.97 (dd) Hb: 2.86 (dd)	7.3, 14.7	43.4 (27), 4.6 (30)
27	122.53	121.04	5.00 (m)	5.00 (m)		43.4 (26), 3.3 (30)
28	133.60	134.81				42.2 (29)
29	17.84	17.74	1.61 (s)	1.64 (s)		42.2 (28)
30	26.05	25.92	1.55	1.63		
31	30.69	30.98	Ha: 2.32 (dd) Hb: 2.41 (dd)	Ha: 2.45 (dd) Hb: 2.35 (dd)	6.4, 15.0	44.4 (32), 3.3 (35)
32	120.86	120.39	4.87 (m)	5.00 (m)		44.8 (31), 3.2 (35)
33	134.71	135.11				42.2 (34)
34	18.25	18.28	1.59 (s)	1.68 (s)		42.2 (33)
35	26.15	26.08	1.53	1.64		
36		168.41				
37		20.55		2.26 (s)		

^a signal multiplicities not determined due to signal overlapping.^b ¹³C-¹³C couplings were detected in the ¹³C NMR spectrum of 2 from the experiment with [U-¹³C₆]glucose.

Table S2. Observed NMR connectivities of 2-acetyl hyperforin (2)

Position	Experiment			
	HMBC	NOESY	COSY	INADEQUATE
1	8a, 8b, 31a, 31b, 32(w)			
2	8a, 8b, 26a, 26b, 31a, 31b(w)			
3	26a, 26b			
4	26a, 26b			
5	14, 15b			
6	8b, 14, 15b			14
7	8a, 8b, 14		8a, 8b, 21a, 21b	8
8	21a, 21b, 31a, 31b	8a(8b), 8b(8a), 14(8a), 31a(8a), 31b(8b)	8a(8b), 8b(8a), 7	7
9	8b, 31a, 31b			
10	11, 12, 13			
11	12, 13	12, 13, 26a	12, 13	12
12	11, 13	11	11	11
13	11, 12	11	11	
14		8a, 15b, 15a(w), 16a, 16b		6
15	14, 16a, 16b	15a(15b), 15b(15a), 14(15b)	15a(15b), 15b(15a), 16a, 16b	
16		14, 16a(16b), 16b(16a), 19	16a(16b), 16b(16a), 15a, 15b, 17	17
17	15a(w), 15b(w), 16a, 16b(w), 19, 20	20	16a, 16b, 19, 20	16
18	16a, 16b(w), 19, 20			19
19	17, 20	16a(w), 16b(w)	17	18
20	17, 19	17	17	
21	7, 8a, 8b, 22	21a(21b), 21b(21a)	21a(21b), 21b(21a), 7, 22	22
22	21a, 21b, 24, 25	25	21a, 21b, 24	21
23	21a(w), 21b(w), 24, 25			24
24	22, 25		22	23
25	22, 24	22		
26	27	26a(26b), 26b(26a), 27(26a,26b)	26a(26b), 26b(26a), 27	27
27	26a, 26b, 29, 30	30, 26a, 26b	26a, 26b	26
28	26a, 26b, 29, 30			29
29	27, 30			28
30	27, 29	27		
31	8a, 8b, 32	8a(31a), 8b(31b), 31a(31b), 31b(31a), 32(31a,31b), 34(31a,31b)	31a(31b), 31b(31a), 32	32
32	31a, 31b, 34, 35	35, 31a, 31b	31a, 31b	31
33	31a, 31b, 34, 35			34
34	32, 35	31a, 31b		33
35	32, 34	32		
36	37			
37				

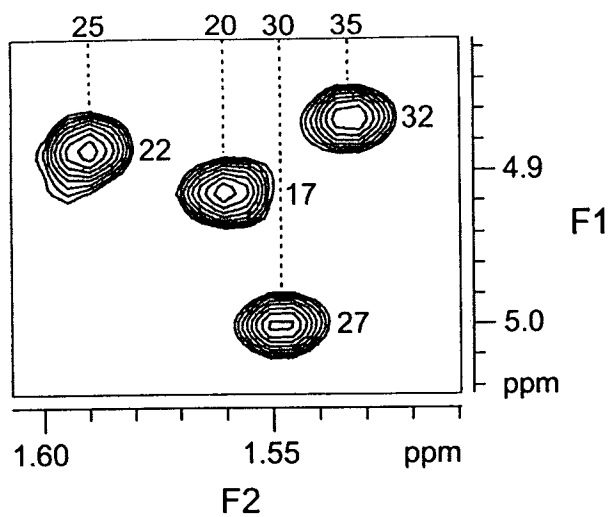


Fig. S1. Part of a two-dimensional homonuclear NOESY spectrum of hyperforin.

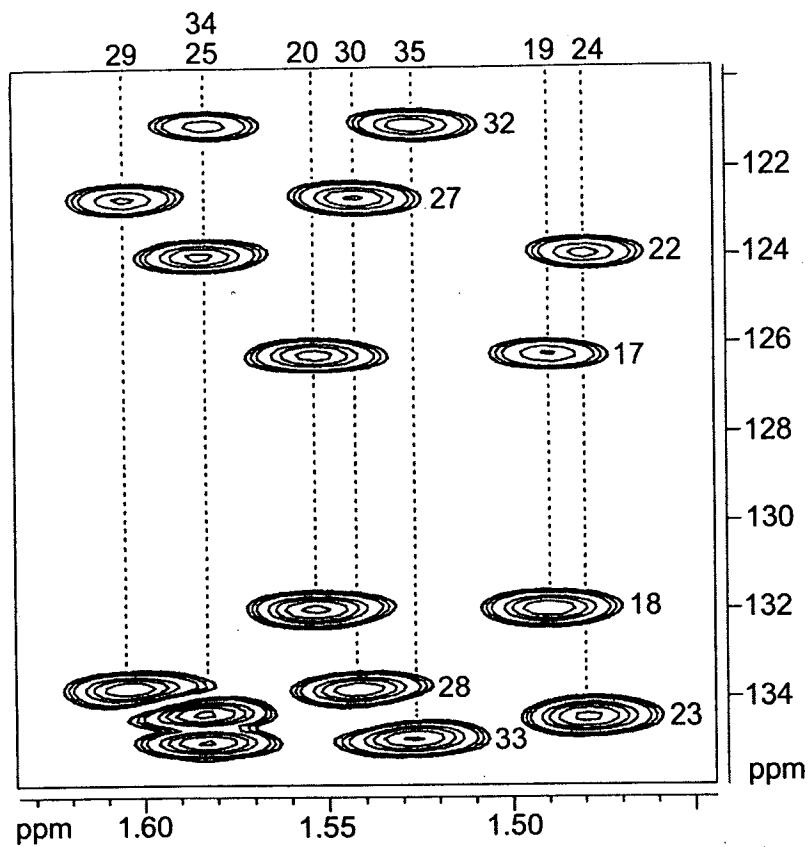


Fig. S2. Part of a two-dimensional ^1H - ^{13}C correlation experiment *via* multiple bonds (HMBC) of hyperforin.