Synthesis and new skin-relevant properties of the salicylic acid ester of bakuchiol

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Abstract

Bakusylan (bakuchiol salicylate) is a bipartite compound obtained by merging two skin-active entities with complementary bioactivities - bakuchiol and salicylic acid - for the purpose of generating a new class of functional retinoids with enhanced skin benefits. Here, we describe its preparation process and report that pure bakusylan exhibits potential for an improved permeation through the stratum corneum, enhances type IV collagen gene expression in organotypic skin substitutes containing both epidermal and dermal layers, and upregulates this protein in adult human dermal fibroblast cultures. The mechanism of action underlying these effects appears to involve the components of the IP3K/Akt signaling pathway selectively implicated in the maintenance of skin integrity, further underlying the suitability of this ester for skin care applications requiring enhanced cutaneous permeation, targeting the dermal-epidermal junction.

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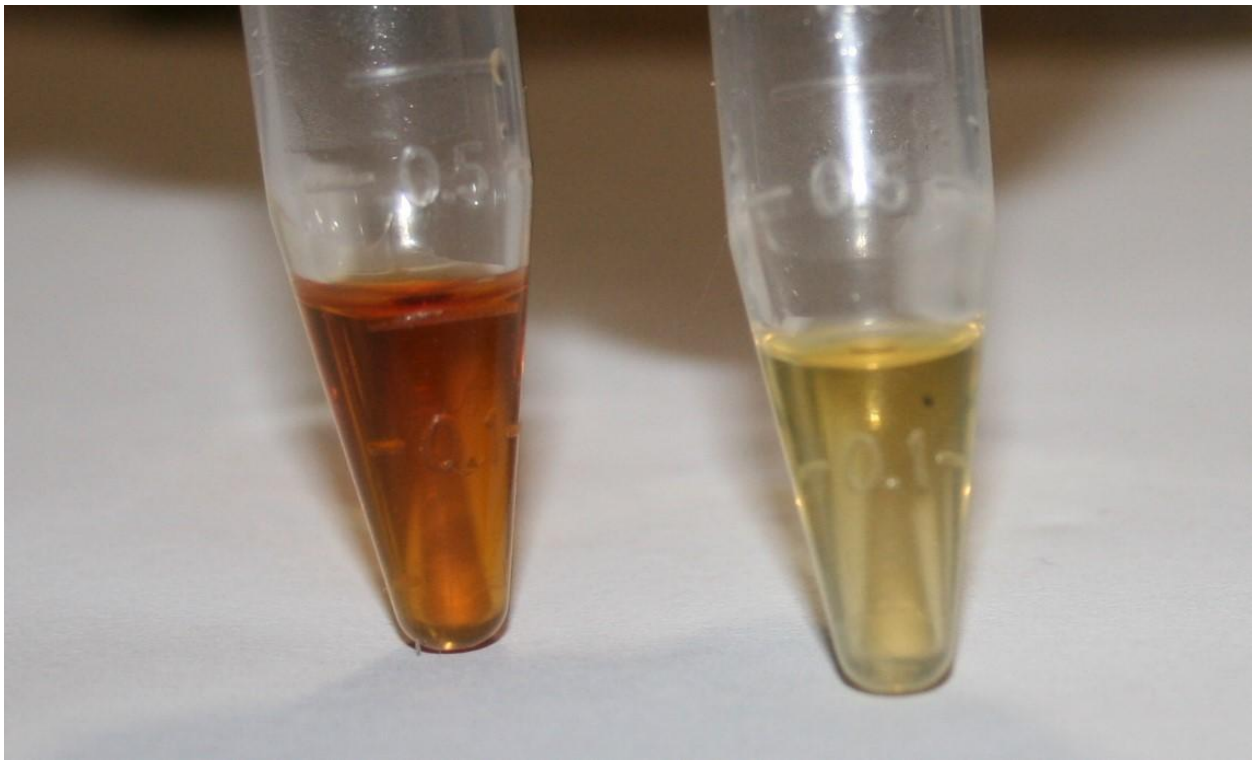
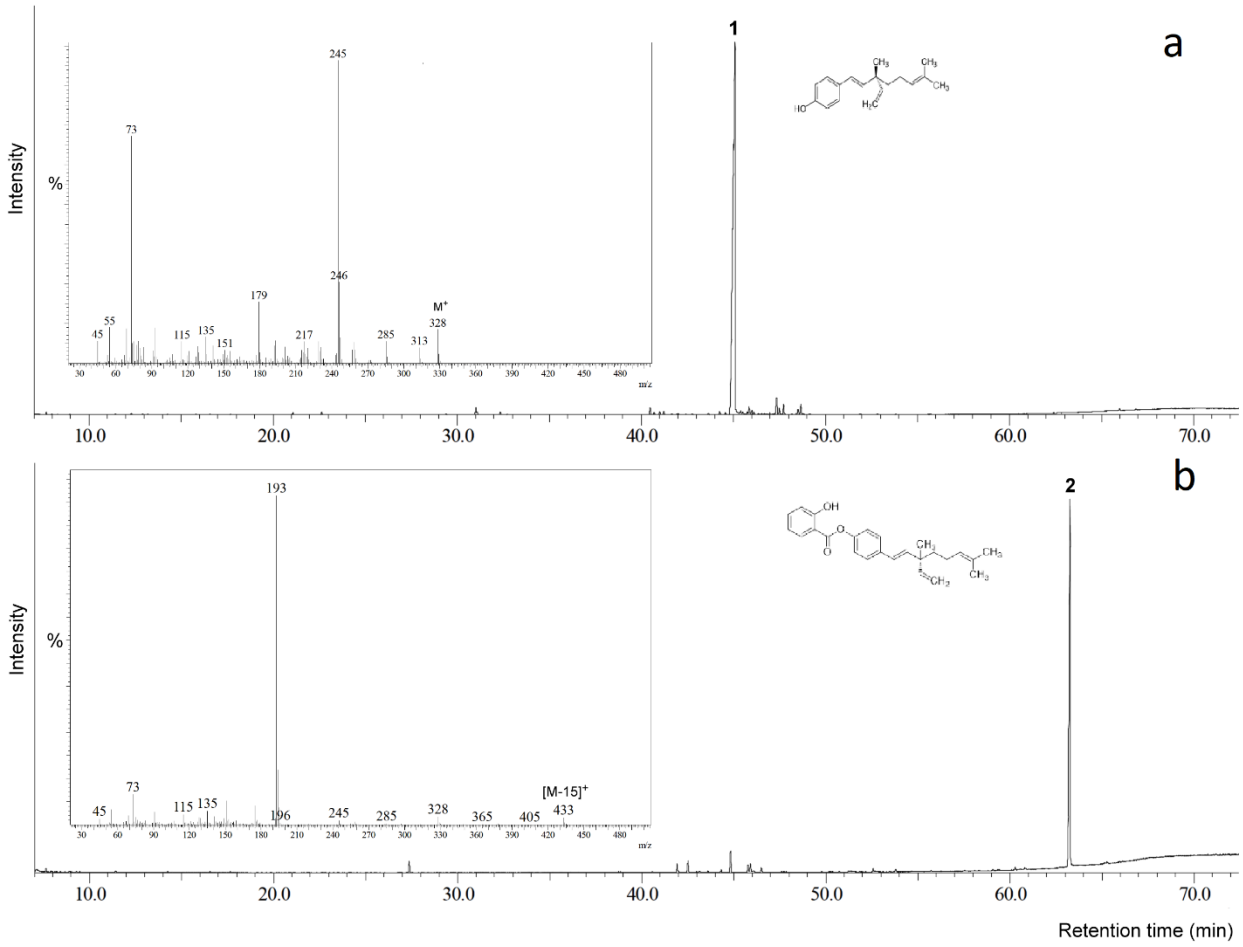
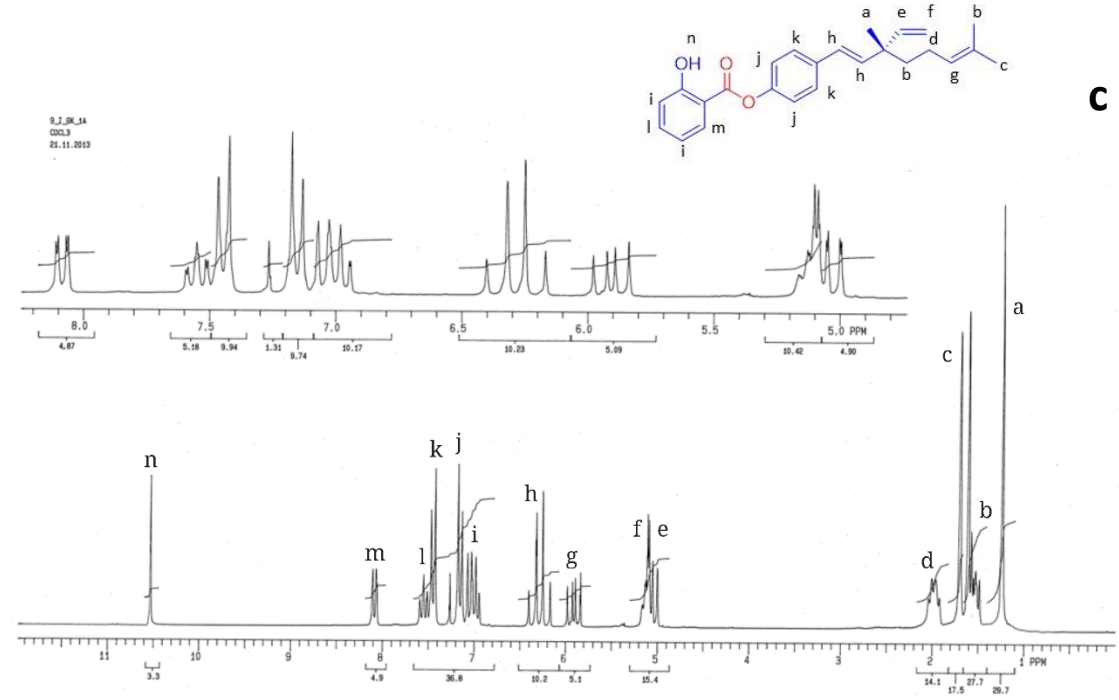


Figure S1. Photographic documentation of the starting material (bakuchiol 75% pure; left) and the purified product (98% purified bakuchiol, right).


a
1
Intensity
%
245
73
246
179
M+
328
55
45
115
135
151
217
285
313
10.0
20.0
30.0
40.0
50.0
60.0
70.0
b
2
193
73
[M-15]+
45
115
135
196
245
285
328
365
405
433
Retention time (min)


c
OH
O
a
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i
8.0
7.5
7.0
6.5
6.0
5.5
5.0 PPM
n
k
j
h
m
l
i
g
f
e
a
c
b
d
1 PPM

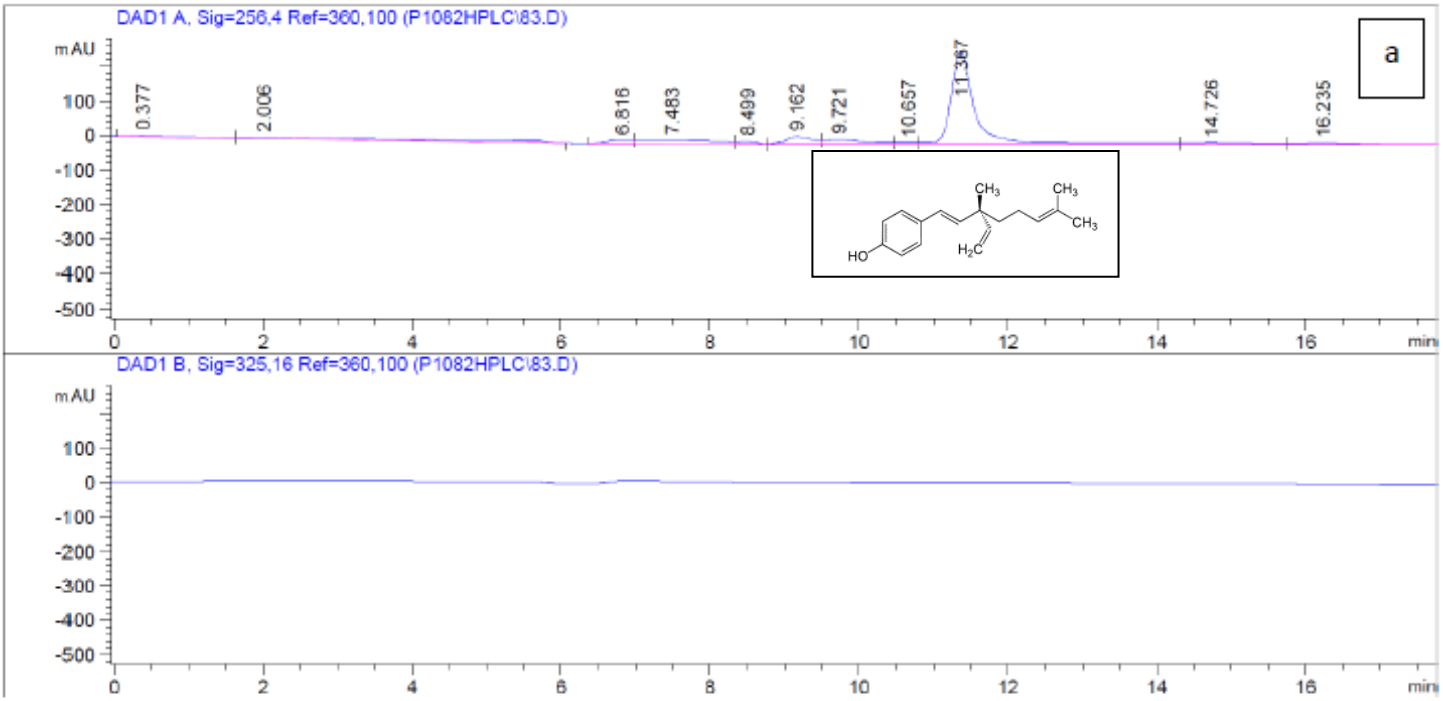
Figure S2. Gas chromatograms from GC-MS analysis of purified bakuchiol (a), and bakusylan (b), as their TMSi derivatives. Inserts: mass spectra of substances in peaks 1 (bakuchiol) and 2 (bakusylan); (c): NMR spectrum of bakusylan; ^{1}H NMR (200 MHz, $CDCl_3$): 1.23 (s, 3H, CH_3), 1.24-1.27 (m, 2H, CH_2), 1.60 (s, 3H, CH_3), 1.70 (s, 3H, CH_3), 1.98 (q, 2H, CH_2, J = 8 Hz), 5.00-5.13 (m, 3H, 3CH), 5.84-5.98 (m, 1H, CH), 6.20 (d, 1H, CH, J = 14 Hz), 6.37 (d, 1H, CH J = 16 Hz), 6.94-7.07 (m, 2H, ArH), 7.15 (d, 2H, ArH, J = 9Hz), 7.44 (d, 2H, ArH, J = 9 Hz), 7.51-7.55 (m, 1H, ArH), 8.08 (dd, 1H, ArH, J_1 = 6 Hz, J_2 = 2H), 10.53 (br s, 1H, OH + D_2O exchangeable) ppm.

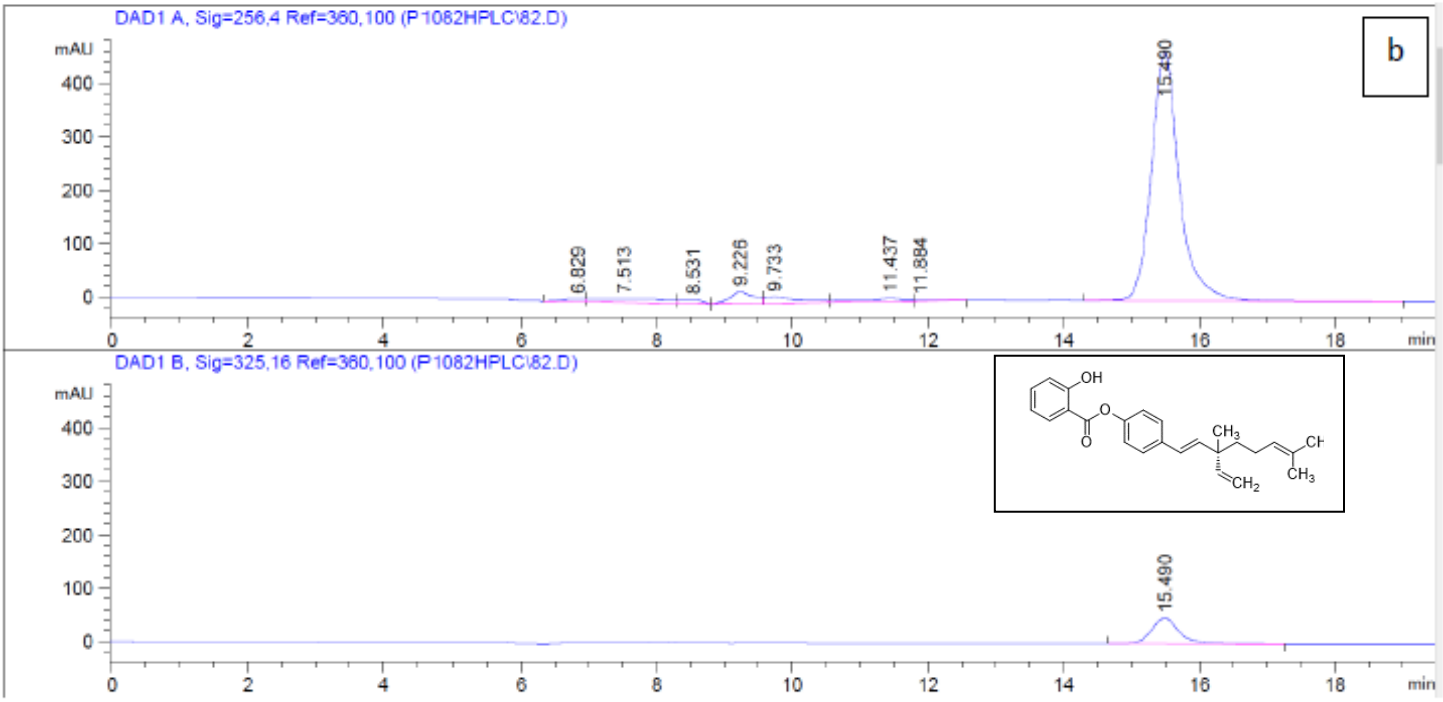
Figure S3. Photographic documentation of the residual bakuchiol (left) and bakusylan (right) after 2h incubation on top of Strat M membrane. Unlike bakuchiol, bakusylan appears to be mostly absorbed by the membrane and thus not readily visible. This agrees with its higher hydrophobicity, and thus, presumably, better ability to blend with lipids, such as in *stratum corneum*, compared to bakuchiol.

Table I. Intramembrane permeation of bakusylan and bakuchiol 2h after topical application on Strat M membrane.

Substance	**Permeation (% of the topically deposited substance)**	**P value**
bakuchiol	4.1	1.000
bakusylan	17	0.018 (vs. bakuchiol)

Figure S4. HPLC chromatograms at peak (256nm) and non-peak (325nm) wavelength of intra-membrane - permeated fractions of bakuchiol (A) and bakusylan (B) applied on top of Strat M membranes for 2h.





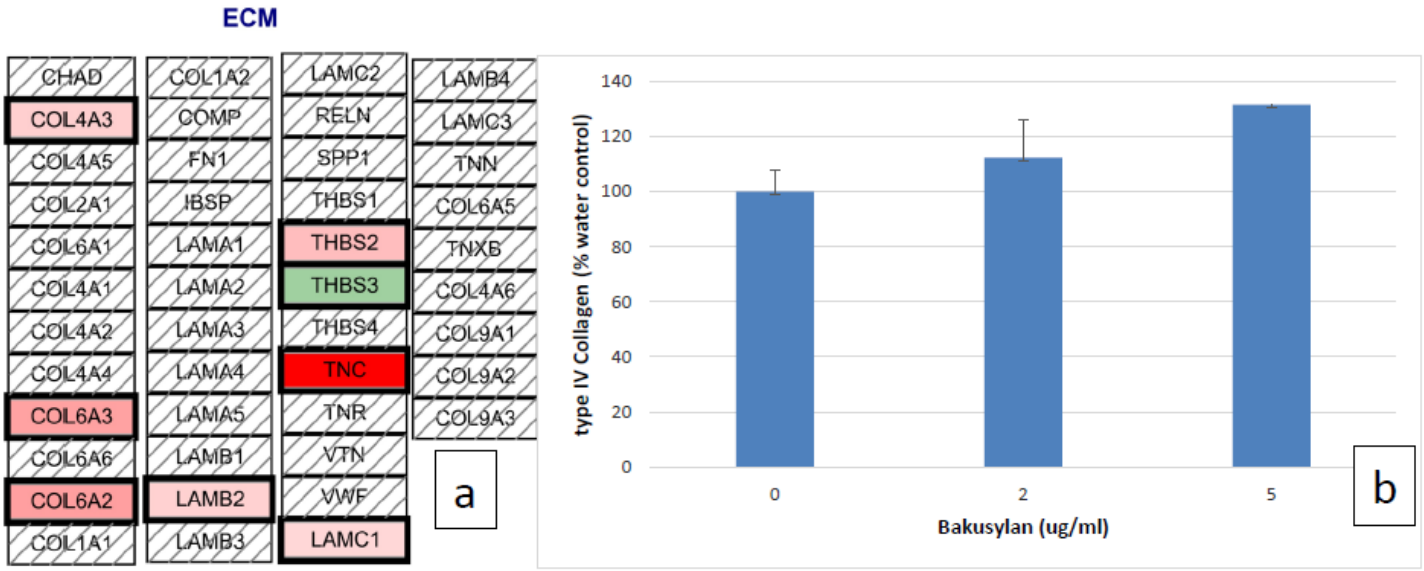


Figure S5. ECM components of the PI3K/Akt signal transduction pathway (WikiPathways). Green and red shaded are genes respectively down- or upregulated by bakusylan in the reconstituted human epidermal tissues, as determined by RNA-seq (darker the color, stronger the modulation). [FC/p values] for the modulated genes: COL4A3: [1.6/0.047]; COL6A2: [2.1/0.038]; COL6A3: [2.1/0.021]; THBS2: [1.8/0.0.014]; THBS3: [-2.1/0.032]; TNC: [4.3/0.0193]; LAMB2: [1.5/0.032]; LAMC1: [1.5/0.049]; **B**: Dose-dependent upregulation of type IV collagen output in the aHDF-conditioned medium (standardized to the overall cellular metabolism). The upregulation by 5µg/ml bakusylan is statistically significant vs. water (solvent) control (132%; p=0.004). Bars represent standard errors of the mean (SEM).