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J. Nat. Prod., **1994**, 57 (5), 654-657• DOI: 10.1021/np50107a016 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 5, pp. 654-657, May 1994

SB-202742, A NOVEL β-LACTAMASE INHIBITOR ISOLATED FROM *SPONDIAS MOMBIN*

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ABSTRACT.—SB-202742 [**1**], an anacardic acid derivative possessing β-lactamase inhibitory activity, has been isolated from a hexane extract of the plant, *Spondias mombin*. Its isolation, structure determination, and biological activity are reported herein.

β-Lactamase inhibitors are a clinically important class of compounds capable of protecting β-lactam antibiotics from inactivation by bacterial β-lactamases. Screening for inhibitors of this type has been carried out extensively in Actinomycetes, and has resulted in the discovery of clavulanic acid (1) and numerous carbapenem antibiotics (2). However, only rarely were inhibitors of a non-β-lactam structure (3–6) isolated from this group of organisms. Therefore, in the hope of discovering inhibitors of a novel structural type, we turned our attention to plant extracts. Such extracts have previously been shown to possess some β-lactamase inhibitory activity (7), although pure inhibitors have rarely been isolated (8). We now wish to report the isolation, structure determination, and biological properties of SB-202742, an anacardic acid derivative extracted from *Spondias mombin* L. (Anacardiacae).

A hexane extract of the leaves and twigs of *S. mombin* exhibited a positive response when tested in a plate bioassay designed to detect β-lactamase inhibitory activity. Purification by sequential bioassay-guided fractionation on Diaion HP20, HP20ss, Si gel, and reversed-phase semi-prep. hplc afforded the active component, SB-202742 [**1**], as a colorless oil.

The molecular formula of SB-202742 was established as $C_{24}H_{34}O_3$ by hreims of the molecular ion (M^+) at *m/z* 370.2505 (calcd 370.2508), while eims of a deuterated sample gave an M^+ at *m/z* 372 indicating the presence of two exchangeable protons. The ^{1}H-nmr spectrum showed signals characteristic of a 1,2,3-trisubstituted phenyl moiety as well as 23 aliphatic and 6 olefinic protons. Confirmation of the existence of 24 carbons in the molecule was demonstrated by the ^{13}C-nmr spectrum, and spectral editing using the DEPT sequence indicated 4 non-protonated, 9 methine, 10 methylene, and 1 methyl resonance. Of the four non-protonated carbon atoms, three were associated with the aromatic ring while the remaining one (δ 176.5) was assigned as a carboxyl carbonyl group. The aromatic ring and carbonyl group accounted for five of the eight degrees of unsaturation apparent from the molecular formula. The remaining three could be attributed to three olefinic double bonds, each associated with two methine protons. A ^{13}C-^{1}H COSY experiment allowed unambiguous assignment of the ^{13}C-nmr spectrum (Table 1) and clearly showed that 6

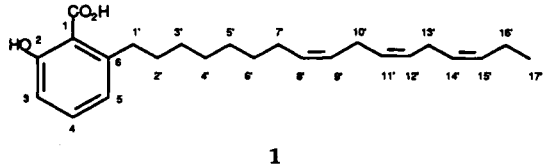

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TABLE 1. ^{13}C- (100 MHz) and ^{1}H- (400 MHz) Nmr Spectral Data for SB-202742 in CD_3OD.

Position	δ_C[a]	δ_H (multiplicity, coupling)
1	118.6 (s)	—
2	162.3 (s)	—
3	115.2 (d)	6.65 (dd, J=7.9 and 1.0 Hz)
4	132.4 (d)	7.11 (dd, J=7.7 and 7.9 Hz)
5	122.5 (d)	6.61 (dd, J=7.7 and 1.0 Hz)
6	147.5 (s)	—
7	176.5 (s)	—
1'.....	36.4 (t)	3.02 (t, J=7.7 Hz)
2'.....	33.3 (t)	1.58 (m)
3'.....	30.1 (t)	1.31 (m)
4'.....	30.8 (t)	1.31 (m)
5'.....	30.6 (t)	1.28 (m)
6'.....	30.4 (t)	1.26 (m)
7'.....	21.5 (t)	2.06 (m)
8'.....	132.7 (d)	5.36 (m)
9'.....	131.2 (d)	5.35 (m)
10'....	26.4[b] (t)	2.79 (m)
11'....	129.2 (d)	5.31 (m)
12'....	129.2 (d)	5.31 (m)
13'....	26.0[b] (t)	2.79 (m)
14'....	128.7 (d)	5.30 (m)
15'....	128.2 (d)	5.27 (m)
16'....	28.2 (t)	2.07 (m)
17'....	14.7 (q)	0.95 (t, J=7.5 Hz)

[a]Multiplicity determined by DEPT.
[b]The δ_C for these signals may be interchanged.

of the 9 methines were olefinic, while the remaining 3 exhibited one-bond C-H correlations with the 1,2,3-trisubstituted phenyl group.

The aromatic substitution pattern was deduced by a consideration of the ^{1}H- and ^{13}C-nmr chemical shifts, while a ^{1}H-^{1}H COSY spectrum suggested the partial structures depicted in Figure 1.

It was apparent from a resolution-enhanced ^{1}H-nmr spectrum that the methylene terminal of partial structure B

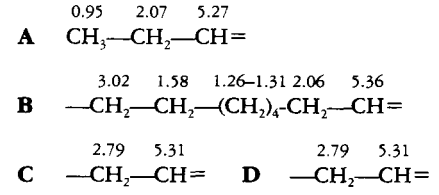

FIGURE 1. Partial Structures and Chemical Shifts (δ_H) Obtained from the ^{1}H-^{1}H COSY Spectrum of SB-202742.

was attached to the aromatic ring by virtue of a small coupling (<0.1 Hz) between it and H-5. The methylene termini in partial structures C and D displayed ^{1}H-nmr chemical shifts which were typical of bis-allylic methylenes. This functionality can only be accommodated by placing these groups between the three olefinic double bonds previously identified. Thus, the structure of SB-202742 was assigned as the known anacardic acid 6-(heptadecatrien-8(Z),11(Z),14(Z)-yl)-2-hydroxybenzoic acid [**1**], previously isolated from *Philodendron scandens* (Araceae) (9) and *Rhus javanica* (Anacardiacae) (10).

The cis geometry for the three olefinic bonds was indicated by virtue of the ^{13}C-nmr chemical shifts of the two bis-allylic methylene carbons at δ 26.0 and δ 26.4; equivalent trans stereochemistry would be indicated by a downfield shift of approximately 10 ppm (11). This stereochemistry is in accord with all previously isolated anacardic acid derivatives (12) and the closely related pelandjuaic acid (13), which are reported to have exclusive cis geometry.

The inhibition of a range of β-lactamases by SB-202742 is illustrated in Table 2. Against the two most clinically important enzymes represented here, SB-202742 had an IC_{50} of 5.0 μg/ml against TEM-1, but no inhibitory activity against the *Staphylococcus aureus* enzyme. Class 1 β-lactamases and PSE4 showed a significant level of inhibition by SB-202742. Pre-incubation of TEM-1 with SB-202742 did not improve the IC_{50} values, showing that it is a reversible inhibitor. Eadie-Hofstee plots demonstrated that SB-202742 was a non-competitive inhibitor of nitrocefin hydrolysis by TEM-2 β-lactamase, with nitrocefin at a range of concentrations between 25 and 400 μM. In bioassay plates as described for the monitoring of SB-202742 extraction, with the concentration of penicillin G elevated to 20 μg/ml for maximum sensitivity, the minimum detect-

TABLE 2. β-Lactamase-Inhibitory Activity of SB-202742.[a]

Enzyme	Class[b]	SB-202742	Clavulanic acid
		IC_{50} μg/ml	IC_{50} μg/ml
Escherichia coli K12 (PSE4)	2c	10.1	0.015
E. coli K12 (OXA1)	2d	79.7	0.35
E. coli JT4 (TEM-1)	2b	5.0	0.029
Pseudomonas aeruginosa A	1	40.5	740
Enterobacter cloacae P99	1	16.5	155
Proteus mirabilis C889	2a	111.3	0.019
Staphylococcus aureus Russell	2a	NI[c]	0.03

[a]IC_{50}s were determined using a Luminar Bio Tek EL312 microtiter plate reader measuring inhibition of nitrocefin (200 μM) hydrolysis. All determinations were made following pre-incubation of inhibitor with enzyme for 5 min at 37°.

[b]See Bush (14,15).

[c]NI=no inhibition at up to 567 μg/ml.

able concentration of SB-202742 was 1 μg/ml. However, in whole cell experiments, SB-202742 at a concentration as high as 100 μg/ml did not display a useful level of synergy with amoxycillin against a range of β-lactamase-producing bacteria, possibly due to poor penetration through the bacterial cell wall.

SB-202742 also displayed weak antibacterial activity against Gram-positive organisms, with MIC values for *Staphylococci* spp. in the 2–32 μg/ml range (Table 3).

The structure-activity relationships of some synthetic and semi-synthetic analogues of SB-202742 will be published elsewhere (16).

TABLE 3. Antibacterial Activity of SB-202742.[a]

Organism	MIC (μg/ml)
Escherichia coli NCTC10418	>256
Pseudomonas aeruginosa 1771P	>256
Proteus mirabilis C977	>256
Enterobacter cloacae N1	>256
Klebsiella pneumoniae A	>256
Serratia marcescens US32	>256
Bacillus subtilis ATCC 6633	4
Staphylococcus aureus Oxford	8
S. aureus Russell	32
S. aureus Smith	2
S. saprophyticus FL2	2
S. epidermidus NCTC 11047	2
Streptococcus faecalis 1	0.25
S. pyogenes CN 10	2
S. pneumoniae PU7	2

[a]The microtiter method was used with Oxoid nutrient broth No. 2 for all organisms except *S. pyogenes* and *S. pneumoniae*, for which Todd Hewitt broth was used. Inocula were a 1/500 dilution of an overnight broth culture. Incubation was at 37° overnight.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Diaion HP 20 (styrene divinyl C_6H_6 cross-linked polymeric adsorbent) was supplied by Mitsubishi Chemical Industries Ltd., Tokyo, Japan. Si gel (Kieselgel 60, 230–400 mesh) was obtained from Merck (Art. No. 9385). Hplc was carried out on a Waters C18 Z Module (0.8×10 cm), using a Waters 600 multisolvent delivery system. Monitoring was by a Waters Lambda Max Model 481 lc spectrophotometer at 310 nm. Eims was recorded on a VG Trio-2 single quadrapole mass spectrometer and hreims on a VG ZAB 1F double-focussing mass spectrometer. Uv and ir spectra were recorded on Pye Unicam SP7-500 and Perkin Elmer 1600 Series Ft-ir spectrophotometers, respectively. The ^1H- and ^{13}C-nmr spectra were measured at 27° on a Bruker AM 400 spectrometer, equipped with a 5 mm $^1H/^{13}C$ dual probe. The TEM-2 β-lactamase preparation used for bioassay was supplied by the Microbiological Research Establishment, Porton, Wiltshire, UK. This was an ammonium sulphate precipitate of cell extracts of *Escherichia coli* W3110 which was further purified as described by Reading and Farmer (17).

PLANT MATERIAL.—*Spondias mombin* was collected in Aburi, Ghana in July 1989 (rainy season) and December 1991 (dry season); a voucher specimen, BIO 031, is held by Prof. R. Thomas, Biotics Ltd., University of Sussex, Brighton, UK. The

extract was prepared from dried pulverized leaves and twigs of *S. mombin* (1 kg) by extraction with hexane.

ISOLATION AND PURIFICATION.—The crude hexane extract (5.2 g) was chromatographed on Diaion HP20 eluting with a stepwise gradient of MeOH-H_2O (40:60) to (100:0), followed by a stepwise gradient of Me_2CO-H_2O (40:60) to (100:0). The active fractions from the 100% MeOH and the 90% and 100% Me_2CO eluates were combined and evaporated to dryness. The residue was dissolved in MeOH-H_2O (80:20) and chromatographed on Diaion HP20ss eluting with MeOH-H_2O (80:20) followed by a stepwise gradient of Me_2CO-H_2O (80:20) to (100:0). The 90% and 100% Me_2CO eluates containing the active material were combined and evaporated, and the residue was chromatographed on Si gel 60 (Merck, 230–400 mesh) eluting with a stepwise gradient of MeOH-$CHCl_3$ (2:98) to (5:95). The combined active fractions were evaporated to dryness and the resulting material was further purified by reversed-phase hplc (Waters C18, 8×100 mm Z module, 20×1 ml injections) with 80% aqueous MeOH at a flow rate of 4 ml/min. SB-202742 [**1**] (160 mg, 3.1% yield) had a R_t of 5.5 min under these conditions.

β-LACTAMASE INHIBITION ASSAY.—Fractions were monitored by plate bioassay on DST agar containing penicillin G (5 μg/ml) in conjunction with a TEM-2 β-lactamase preparation (0.0025 μg/ml), using *Bacillus subtilis* ATCC 6633 as the assay organism. The plate was incubated at 37°, and fractions containing β-lactamase inhibitor were identified by a zone of bacterial inhibition due to the presence of intact penicillin.

SB-202742 {6-[heptadecatrien-8(Z),11(Z),14(Z)-yl]-2-hydroxybenzoic acid} [**1**].—A colorless oil: ir ν max ($CHCl_3$) 3011, 2932, 2856, 1647, 1608, 1448 cm^{-1}; uv λ max (MeOH) 205 (ε 22497), 241 (ε 3532), 308 (ε 2160) nm; hreims *m/z* [M^+] 370.2505 (calcd 370.2508 for $C_{24}H_{34}O_3$); eims *m/z* [M^+] 370 (20), 352 (6), 337 (16), 326 (30), 147 (62), 133 (43), 121 (54), 108 (84), 95 (75), 79 (100), 67 (72), 55 (60); ^1H and ^{13}C nmr see Table 1.

ACKNOWLEDGMENTS

The authors wish to thank E.A. Cutmore, G.D. Risbridger, D.A. Tolson, and J. White for spectroscopic data. We also express our thanks to Biotics Ltd., University of Sussex, Brighton, BN1 9Q J, UK for the supply of plant extracts.

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Received 6 October 1993