

A LIMONOID ANTIFEEDANT FROM SEED OF *CARAPA PROCERA*¹

K.L. MIKOLAJCZAK,* D. WEISLEDER,

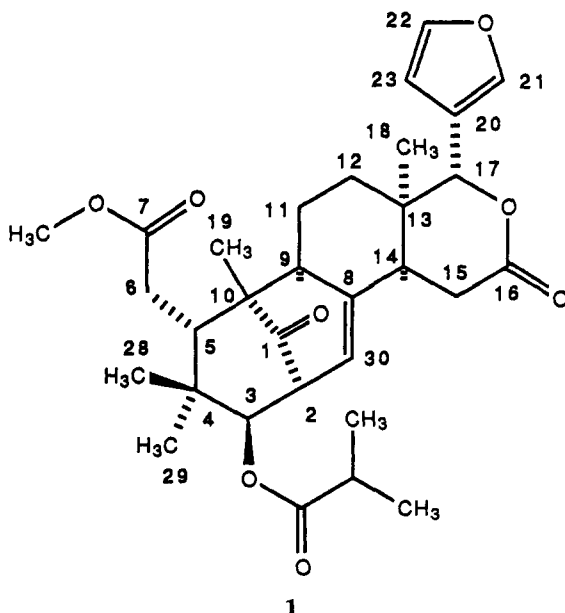
Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture,
1815 North University Street, Peoria, Illinois 61604L. PARKANYI,² and J. CLARDY*

Department of Chemistry, Cornell University, Ithaca, New York, 14853

We have recently been investigating some selected, lesser known species of the family Meliaceae in a search for new natural products with activity in insects. Effects of their seed extracts on fall armyworm larvae [*Spodoptera frugiperda* (J.E. Smith)] (Lepidoptera: Noctuidae) (1,2), and on striped cucumber beetle adults [*Acalymma vittatum* (F.)] (Coleoptera: Chrysomelidae) (1) have been compared with effects produced by comparable neem seed (*Azadirachta indica* A. Juss.) extracts.

In these studies, the hexane extract of

seeds from one of these species, *Carapa procera* DC., inhibited feeding by the fall armyworm in a choice leaf disk bioassay to the same degree that neem seed hexane extract did. However, all larvae fed artificial diets containing neem seed hexane extract in a no-choice test died, but larvae that were fed *C. procera* extract survived to pupation. In addition, the *C. procera* extract, unlike the neem extract, did not deter feeding by striped cucumber beetles in leaf disk assays (1). Because the observed differences in activity presented the possibility that



¹The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

²Permanent address: Central Research Institute for Chemistry of the Hungarian Academy of Sciences, Budapest, Hungary.

something other than azadirachtin was responsible, we undertook the isolation of the active material and the determination of its structure.

Compound A was isolated from *C. procera* seed oil by a series of chromatographic procedures, and its structure

was found to be **1**; its X-ray-derived structure is shown in Figure 1. Hydrogens are omitted for clarity, and the absolute configuration was arbitrarily selected to agree with that usually found for the limonoids. The [3.3.1] bicyclic nonane contains two embedded six-membered rings. The left-hand ring is in a twist-boat conformation, and the right-hand ring is in a half-chair conformation. The remaining six-membered rings are both in the chair conformation. The C-13–C-14 fusion is *cis*. The furan ring occupies an equatorial position on the lactone ring. In general, all bond distances and angles agree well with generally accepted values. ^1H - and ^{13}C -nmr, 2D proton homonuclear correla-

tion, and ms data all support structure **1**.

Although not previously known in *Carapa*, compound A has been isolated previously from the meliaceous plants *Khaya senegalensis* and *Khaya nyasica* and was named methyl 3 β -isobutyryloxy-1-oxomeliac-8(30)-enate (3). When compound A was presented to the fall armyworm in the antifeedant bioassay, a feeding ratio of 0.03 was obtained.

A number of limonoid compounds have been reported previously from *Carapa* species (4–9), and some, like A, are members of the mexicanolide group (9). No particular compound isolated to date has been shown to possess activity in insects, but Jacobson (10) says of *C. procera*, "The seeds are poisonous and

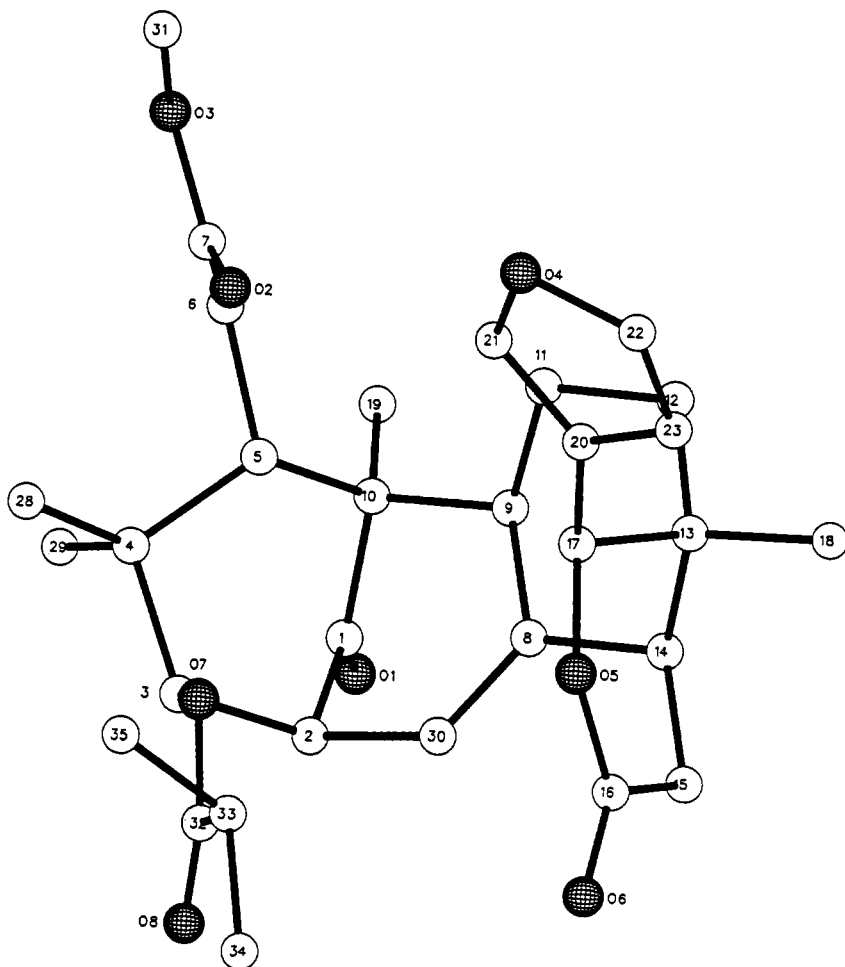


FIGURE 1. The crystal structure of compound A [1].

contain a fat used to kill jiggers in West Africa." Also, indications are that *Carapa guianensis* has some antitermitic properties (11).

EXPERIMENTAL

PLANT MATERIAL AND EXTRACTION.—Seeds of *C. procera* DC., stored at NRRRC, Peoria, Illinois, under the identifier NU61772, were collected in Ghana and authenticated by USDA botanists, Beltsville, Maryland. A sequential extraction of the ground seed with *n*-hexane and then with 95% EtOH was done in a Soxhlet apparatus for 7 h each. Solvent was removed with a rotary evaporator at a bath temperature of 40°. The yield of recovered hexane extract was 37%, and that of EtOH extract was 3%.

ANTIFEEDANT BIOASSAY.—A modified version of the leaf disk bioassay (2, 12) was used. The disposable Petri dish arenas were 150 × 15 mm; six treated and six control green bean leaf disks (1-cm diameter) were arranged alternately around the circumference. Three 7- to 9-day-old fall armyworm larvae that had been starved 24 h were introduced into each arena (3 replicates) and were allowed to feed for 3 h. A feeding ratio was determined by dividing the mean percent of sample-treated disks consumed by the mean percent of control disks consumed; a value of 0.25 or less was arbitrarily set as being indicative of strong feeding deterrence due to the test material.

FRACTIONATION OF EXTRACT.—*C. procera* hexane extract (40.8 g) was partitioned between 500 ml each of hexane and MeOH-H₂O (9:1), and 3.5 g of active material was recovered from the aqueous MeOH phase. The hexane solubles were inactive. Preparative hplc of the active fraction on a 1.0 × 50-cm silica column with EtOAc-hexane (1:9) produced three active fractions that were recombined. This material was then further resolved on a 0.47 × 25-cm C-18 reversed-phase hplc column with H₂O-MeOH (3:7). Preparative tlc of the resulting active fractions was done on 2-mm silica plates with an Et₂O-hexane-HOAc (55:45:1) solvent system. A final tlc purification on analytical (0.25 mm) silica plates produced 48 mg of active constituent A containing minor amounts of impurity. All fractionation steps were monitored by tlc, and most were checked by bioassay as well.

By dissolving A in 1.0 ml of Et₂O, allowing the solvent to evaporate slowly at -20°, recovering the crystals, and repeating the process, pure compound A (14 mg) was obtained.

COMPOUND A [1].—C₃₁H₄₀O₈, mp 211–213°. Mass spectra were obtained with a Finnigan MAT 4535/TSQ instrument: ms (70 eV) *m/z* (rel. int.) [M]⁺ 540 (23), 437 (8), 402 (7), 363 (10),

175 (18), 121 (11), 105 (11), 95 (52), 91 (14), 71 (36), 43 (100).

NMR DATA FOR COMPOUND A [1].—Nmr spectra were obtained with a Bruker WM-300 WB spectrometer. A 5-mm dual (¹H/¹³C) probe was used to obtain ¹H, ¹³C, edited DEPT (13), and 2D proton homonuclear correlation spectra (14). ¹H nmr (300 MHz, CDCl₃) δ 3.49 m (H-2, *J*_{2,3} = 9.4 Hz, *J*_{2,30} = 7.3 Hz), 4.76 (H-3, d), 3.40 (H-5, dd, *J*_{5,6} = 7.4 Hz, *J*_{5,6'} = 4.4 Hz), 2.36 (H-6, 6', m), 2.10 (H-9, m), 1.67 (H-11, 12, m), 2.22 (H-14, m, *J*_{14,15'} = 5.9 Hz), 2.80 (H-15, m), 2.90 (H-15', m), 5.68 (H-17, s), 1.13 (H-18, s), 0.81 (H-19, s), 7.78 (H-21, br s), 6.45 (H-22, d), 7.40 (H-23, m), 1.08 (H-28, s), 0.78 (H-29, s), 5.35 (H-30, br d), 3.70 (-OMe, s), 1.15 (Me₂CH-, d), 1.16 (Me₂CH-, d), 2.61 (Me₂CH-, m, *J*_{CH,ME} = 7.0 Hz); ¹³C nmr (75.5 MHz, CDCl₃) δ 216.9 (C-1, s), 56.8 (C-2, d), 77.1 (C-3, d), 38.7 (C-4, s), 48.8 (C-5, d), 33.0 (C-6, t), 176.5 (C-7, s), 138.4 (C-8, s), 41.4 (C-9, d), 49.9 (C-10, s), 20.6 (C-11, t), 29.9 (C-12, t), 36.9 (C-13, s), 45.3 (C-14, d), 34.5 (C-15, t), 174.0 (C-16, s), 77.1 (C-17, d), 22.5 (C-18, 19, 28, 29, q), 21.9 (C-18, 19, 28, 29, q), 20.5 (C-18, 19, 28, 29, q), 15.8 (C-18, 19, 28, 29, q), 120.7 (C-20, s), 142.9 (C-21, d), 109.7 (C-22, d), 141.9 (C-23, d), 122.8 (C-30, d), 52.1 (-OMe, q), 169.3 (isobutyloxy C=O, s), 19.1 [(CH₃)₂CH-, q], 18.6 [(CH₃)₂CH-, q], 33.9 (Me₂CH-, d).

X-RAY DATA FOR COMPOUND A [1].³—An approximately cubic crystal with edges 0.25 mm was used for all diffraction experiments on a Syntex P₂ diffractometer with graphite monochromated CuKα radiation (1.54178 Å). Crystal data: C₃₁H₄₀O₈, MW = 540.7, orthorhombic space group P2₁2₁2₁, a = 10.322(1), b = 15.316(2), and c = 17.754(1) Å, V = 2806.6(6) Å³, Z = 4, D_c = 1.280 g cm⁻³, μ (CuKα) = 7.10 cm⁻¹, T = 22°. Data were collected with variable speed, 1° ω-scans with 2θ < 114°. Of the 2314 unique data examined, 1981 (81%) were judged observed [F_o > 1.8σ(F_o)] after correction for background, Lorentz, and polarization effects.

The structure was solved by direct methods with the MULTAN package of programs.⁴ Hy-

³Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

⁴MULTAN 80 and RANTAN 80, systems of computer programs for the automatic solution of crystal structures from X-ray diffraction data (locally modified to perform all Fourier calculations including Patterson syntheses) written by P. Main, S.E. Hull, L. Lessinger, G. Germain, J.P.

TABLE 1. Coordinates and Equivalent Thermal Parameters for Compound A [1].

Atom	x	y	z	B
O-1	1.1425 (3)	0.0707 (3)	0.9588 (2)	7.4 (1)
O-2	0.7174 (3)	-0.1259 (2)	0.7484 (2)	6.7 (1)
O-3	0.8666 (3)	-0.2005 (2)	0.6836 (2)	6.5 (1)
O-4	0.4145 (4)	0.0268 (4)	0.6482 (2)	9.7 (1)
O-5	0.5205 (3)	0.1700 (2)	0.8533 (2)	6.1 (1)
O-6	0.4931 (4)	0.2315 (3)	0.9649 (2)	7.8 (1)
O-7	0.7176 (3)	-0.0361 (2)	0.9697 (2)	6.1 (1)
O-8	0.6862 (3)	0.0128 (3)	1.0865 (2)	7.4 (1)
C-1	1.0391 (5)	0.0501 (3)	0.9338 (3)	5.9 (1)
C-2	0.9174 (5)	0.0491 (3)	0.9809 (2)	5.7 (1)
C-3	0.8550 (5)	-0.0425 (3)	0.9838 (2)	5.9 (1)
C-4	0.9096 (5)	-0.1061 (3)	0.9225 (3)	6.6 (1)
C-5	0.9185 (4)	-0.0542 (3)	0.8477 (3)	5.7 (1)
C-6	0.9445 (5)	-0.1125 (3)	0.7792 (3)	6.7 (1)
C-7	0.8287 (4)	-0.1462 (3)	0.7396 (2)	5.7 (1)
C-8	0.8491 (5)	0.1497 (3)	0.8748 (2)	5.3 (1)
C-9	0.9460 (4)	0.1094 (3)	0.8199 (3)	5.9 (1)
C-10	1.0130 (4)	0.0277 (3)	0.8511 (3)	6.0 (1)
C-11	0.8813 (5)	0.0940 (3)	0.7433 (3)	6.4 (1)
C-12	0.8030 (6)	0.1709 (3)	0.7137 (2)	6.6 (1)
C-13	0.7056 (5)	0.2098 (3)	0.7712 (3)	6.2 (1)
C-14	0.7779 (5)	0.2295 (3)	0.8448 (3)	5.9 (1)
C-15	0.6864 (6)	0.2707 (3)	0.9007 (3)	6.6 (2)
C-16	0.5622 (5)	0.2235 (3)	0.9103 (3)	6.1 (1)
C-17	0.6025 (4)	0.1412 (3)	0.7932 (2)	5.6 (1)
C-18	0.6437 (6)	0.2916 (3)	0.7378 (3)	7.6 (1)
C-19	1.1441 (5)	0.0131 (4)	0.8136 (3)	7.1 (1)
C-20	0.5122 (4)	0.1120 (3)	0.7310 (2)	5.8 (1)
C-21	0.5113 (5)	0.0337 (4)	0.6979 (3)	7.7 (2)
C-22	0.3525 (5)	0.1082 (4)	0.6474 (3)	7.2 (1)
C-23	0.4108 (6)	0.1583 (4)	0.6950 (3)	7.3 (2)
C-28	0.8216 (6)	-0.1839 (3)	0.9148 (3)	7.8 (1)
C-29	1.0430 (6)	-0.1408 (4)	0.9509 (3)	7.7 (2)
C-30	0.8330 (5)	0.1204 (3)	0.9446 (2)	5.7 (1)
C-31	0.7668 (6)	-0.2314 (4)	0.6341 (3)	7.0 (2)
C-32	0.6413 (5)	-0.0066 (3)	1.0263 (2)	5.9 (1)
C-33	0.5024 (5)	0.0004 (4)	1.0029 (3)	7.0 (1)
C-34	0.4224 (6)	0.0399 (6)	1.0652 (4)	9.3 (2)
C-35	0.4531 (9)	-0.0855 (7)	0.9818 (8)	13.1 (4)

drogens were included at calculated positions. Block diagonal least squares refinements have converged to a conventional crystallographic residual of 0.055. Coordinates are given in Table 1.

ACKNOWLEDGMENTS

We thank Barry E. Jones for technical support and acknowledge the financial support of the National Institutes of Health and the National Science Foundation.

LITERATURE CITED

Declercq, and M.M. Woolfson, University of York, England, 1980; BLS78A, an anisotropic block diagonal least squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; PLUTO78, a locally modified crystallographic illustration program by W.D.S. Motherwell, Cambridge Crystallographic Data Centre, 1978; and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu and G. Van Duyne, Cornell University, 1987.

1. K.L. Mikolajczak and D.K. Reed, *J. Chem. Ecol.*, **13**, 99 (1987).
2. K.L. Mikolajczak, B.W. Zilkowski, and R.J. Bartelt, *J. Chem. Ecol.* (in press).
3. D.A.H. Taylor, *J. Chem. Soc. C*, 2439 (1969).
4. E.O. Arene, C.W.L. Bevan, J.W. Powell, and D.A.H. Taylor, *J. Chem. Soc. Chem. Commun.*, 302 (1965).

5. A.F. Cameron, J.D. Connolly, and A. Maltz, *Tetrahedron Lett.*, 967 (1979).
6. S.F. Kimbu, J.F. Ayafor, B.L. Sondengam, E. Tsamo, J.D. Connolly, and D.S. Rycroft, *Tetrahedron Lett.*, **25**, 1613 (1984).
7. D. Lavie, E.C. Levy, and R. Zelnik, *Bioorg. Chem.*, **2**, 59 (1972).
8. B.L. Sondengam, C.S. Kamga, S.F. Kimbu, and J.D. Connolly, *Phytochemistry*, **20**, 173 (1981).
9. D.A.H. Taylor, *Prog. Chem. Org. Nat. Prod.*, **45**, 1 (1984).
10. M. Jacobson, "Insecticides from Plants: A Review of the Literature, 1954-1971," USDA Agriculture Handbook No. 461, 1975.
11. S.C. Jones, F.L. Carter, and J.K. Mauldin, *Environ. Entomol.*, **12**, 458 (1983).
12. I. Kubo and K. Nakanishi, in: "Host Plant Resistance to Pests." Ed. by P.A. Hedin, ACS Symposium Series 62, American Chemical Society, Washington, DC, 1977, pp. 165-178.
13. M.R. Bendall and D.T. Pegg, *J. Magn. Reson.*, **53**, 272 (1983).
14. A. Bax and R. Freeman, *J. Magn. Reson.*, **44**, 542 (1981).

Received 2 December 1987