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CELANGULIN: A NONALKALOIDAL INSECT ANTIFEEDANT FROM CHINESE BITTERSWEET, *CELASTRUS ANGULATUS*

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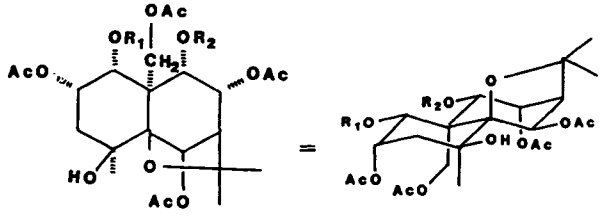
ABSTRACT.—An insect antifeedant, celangulin [**1**], was isolated from Chinese bittersweet, *Celastrus angulatus*. It is a sesquiterpenoid compound that has a dihydroagarofuran skeleton with seven hydroxyl functions, five of which are acetylated, one benzoylated, and one free. Its structure was determined mainly by nmr and ms.

Traditionally, the powdered root bark of Chinese bittersweet, *Celastrus angulatus* Max. (Celastraceae), has been used in China to protect plants from insect damage (1). Investigations of the powdered root bark have demonstrated activity against several insect species. Included are cucumber beetle, *Aulacophora femoralis chinensis* (2), cruciferous leaf beetle, *Colaphellus bowringi* (3), willow leaf beetle, *Plagiodera versicolora* (4), cabbage sawfly, *Athalia flacca* (5), Hawaiian beet webworm, *Hymenia recurvalis* (6), imported cabbageworm, *Pieris rapae* (5), a tent caterpillar, *Malacosoma neustria testacea* (5), and migratory locusts, *Locusta migratoria migratorioides* (3) and *Locusta migratoria manilensis* (5). A review of the chemistry of this family of plants is available (7). Insecticidal alkaloids, such as wilfordine from *Tripterygium wilfordii* Hook (8) and wilforine, an insect antifeedant alkaloid from *Maytenus rigida* (9), have been isolated. The isolation and structure of a new compound, celangulin [**1**], from the Et_2O extract of Chinese bittersweet root bark are reported in this paper. This knowledge could provide the basis for developing more effective insect control chemicals, because celangulin exhibits antifeedant activity using the fall armyworm bioassay.

The presence of a benzoate ester in celangulin was suggested by maxima at 231 and



1 R_1=Ac, R_2=Bz
2 R_1=Bz, R_2=Ac

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281 nm in its uv spectrum [benzoic acid had λ max nm (ε) 229 (20,900) and 281 (1540)]. The ^{13}C-nmr spectrum had 29 peaks (Table 1). No aldehyde or ketone carbonyl carbons were present, but the benzoate carbonyl was at δ 164.7, and four acetate carbonyls were found at δ 169.5, 169.7, 169.9, and 170.5. Acquisition of data with a delay of 20 sec between pulses showed that the acetate carbonyl peak at δ 169.5 was twice as large as the other three peaks; therefore, celangulin had five acetate ester groups. Peaks at δ 20.3, 21.0, 21.2, 21.45, and 21.53, which were assigned to acetate methyl groups, were consistent with the presumption that there were five acetate groups and that the peak at δ 169.5 represented two carbons.

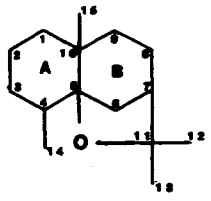
TABLE 1. ^{13}C-nmr (75 MHz) Data for Celangulin [1] in $CDCl_3$.[a]

Carbons	Chemical Shift (δ)
1,2,6,8,9	68.0, 69.9, 72.2, 75.2, 76.1
3	42.1
4	69.8
5	91.9
7	53.4
10	53.1
11	82.9
12,13,14	24.2, 24.4, 29.4
15	60.4
Acetates	
Methyls	20.3, 21.0, 21.2, 21.45, 21.53
C=O	169.5, 169.5, 169.7, 169.9, 170.5
Benzoate 1′	129.6
2′,6′	129.3
3′,5′	128.5
4′	133.6
C=O	164.7

[a]Assignments were made by consideration of literature values (9) and of attached proton tests.

Low resolution eims of celangulin displayed a high ion mass at *m/z* 588 (2.3%) and prominent peaks at *m/z* 202 (35.1%), $[PhCO]^+$ 105 (100%), and $[MeCO]^+$ 43 (43%). Low resolution cims with NH_3 as the reagent gas gave a high mass response at *m/z* 666 that is believed to be the adduct ion $[M + H + NH_3]^+$; therefore, the molecular weight of celangulin was 648. Thus, the highest mass ion detected at *m/z* 588 in eims corresponds to the elimination of HOAc. High resolution eims showed that the molecular formula for the *m/z* 588 peak was $C_{30}H_{36}O_{12}$, leading to the conclusion that the formula for celangulin was $C_{32}H_{40}O_{14}$. Cims of celangulin, with ND_3 as the reagent gas, indicated the presence of a single hydroxyl group by a high mass ion at *m/z* 671 (11).

The ^{13}C-nmr data are brought into agreement with this molecular formula by counting the four aromatic carbon peaks as six carbons and assuming the presence of five acetate groups. As celangulin has one benzoate, one free hydroxyl, and five acetate groups, the unesterified parent compound would be $C_{15}O_{26}O_8$, in which seven of the eight oxygen atoms are in hydroxyl groups and the remaining one presumed to be an ether. Many polyhydroxylated sesquiterpenes based on dihydroagarofuran [3] esterified by one or two benzoic, nicotinic, or furoic acid groups and several HOAc residues have been found in the genus *Celastrus* (7, 12–17). Where ^{13}C-nmr data are reported for these compounds, C-5 has a value between δ 86.3 and 92.4 and C-13 between δ 80.1 and 83.7 (14,18). These peaks appear at δ 82.9 and 91.9, respectively, in celangulin.



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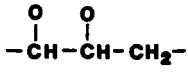
These two ^{13}C-nmr peaks, the molecular formula of the unesterified C_{15} polyol, and the presence of one benzoate and five acetate esters allow the placement of celangulin firmly in the dihydroagarofuran class of natural products.

The absence of a doublet methyl group in the region of δ 0.9–1.3 and the absence of a proton peak between δ 2.8 and 4.8 in the ^{1}H-nmr spectrum (Table 2) were evidence

TABLE 2. ^{1}H-nmr (300 MHz) Data for Celangulin [**1**] in $CDCl_3$.

Hydrogen on carbon	Chemical shift (δ)	Multiplicity	Coupling constant (Hz)
1	5.47	doublet	3.8
2	5.34	doublet of doublet of doublet	2.2, 3.8, 4.3
3	1.94 & 2.20	AB quartet	13.1, 2.2, 4.3
6	6.96	singlet	
7	2.40	doublet	3.7
8	5.58	doublet of doublet	3.7 & 5.9
9	5.65	doublet	5.9
12	1.66	singlet	
13	1.58	singlet	
14	1.49	singlet	
15	4.88 & 5.09	AB quartet	13.5
Acetate	1.48, 2.06, 2.10, 2.14, 2.33	singlets	
Benzoate 2′,6′	8.01	doublet	8.3
3′,5′	7.47	triplet	8.3
4′	7.59	triplet	8.3

that both the C-14 methyl group and the free hydroxyl group were on the same quaternary carbon, C-4. The C-4 hydroxyl group is not esterified in other compounds of this class (7). Decoupling experiments showed that irradiation of the apparent quartet at δ 5.34 (H on C-2) caused the doublet at δ 5.47 (H on C-1) to collapse to a singlet and the smaller splitting (J = 2.2 and 4.3 Hz) of the AB quartet at δ 1.94 and δ 2.20 (2H on C-3) to disappear. This AB quartet must belong to the one methylene group in the rings permitted by the molecular formula; therefore, the partial structure **I** was indicated. Furthermore, this partial structure can only be placed in ring A. The C-2 proton at δ 5.34 was assigned an equatorial conformation because of the absence of axial-axial coupling with the adjacent methylene group. Partial structure **I** is then placed in the dihydroagarofuran skeleton as shown in **1** with the methylene group at C-3 based on the precedent in this class of compounds in which, without exception, an equatorial hydroxyl group is found on C-1 (7). An nOe experiment in which the methyl singlet at δ 1.49 was irradiated resulted in signal enhancement of the proton on C-6 (δ 6.96) and one of the protons on C-15 (δ 5.09), which indicated that the C-14 and C-15 methyl groups and H-6 were axial and affirmed the *trans* ring fusion.



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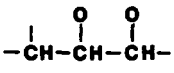
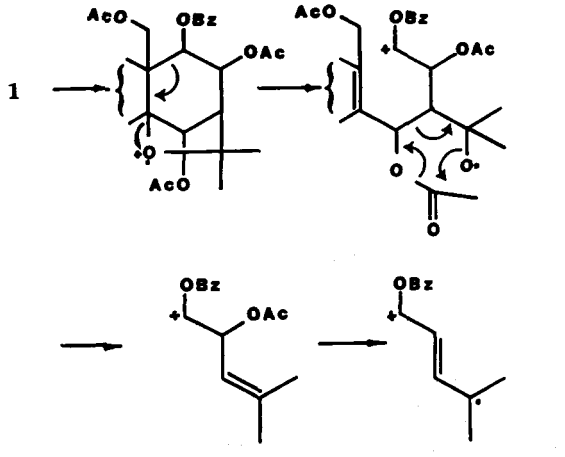


II

Turning to ring B, the singlet at δ 6.96 was assigned to an axial proton at C-6. Such highly deshielded values for the C-6 protons have been reported (18,19). Irradiation of the peak at δ 2.40 (H on C-7) caused the peak at δ 5.58 (H on C-8) to collapse to a doublet and become half an AB quartet with the other half at δ 5.65 (H on C-9). Another partial structure, **II**, may be written and placed unambiguously in ring B, as in **1**. The coupling constants ($J = 3.7$ and 5.9 Hz) closely matched the values reported for an axial proton on C-9 (18–20). Further support came from the fact that an increase in intensity of the C-9 proton was noted when an nOe experiment was carried out in which the methyl singlet at δ 1.66 was irradiated.

The methyl group of an acetate ester normally has a value in ^{1}H nmr in the range of δ 1.9 to 2.7 (21). Four of the five acetate methyls in celangulin fall within this range. There is another one at δ 1.48. Highly shielded methyls such as this occur in this class of compounds, for instance, in maytoline and maytine between δ 1.5 and 1.7 (22), in cathidine D at δ 1.66 (23), in a model compound that was prepared for the structure determination of alatamine at δ 1.52 (8), and in celapanin (δ 1.68), celapanigin (δ 1.67), and celapagin (δ 1.64) (15,16). In all of these compounds, either the C-1 or the C-9 hydroxyl group is esterified with an aromatic carboxylic acid and the other with HOAc. The effect has been attributed to the presence of the anisotropic group in the vicinity of the acetate. Thus, the presence of an acetate methyl group at δ 1.48 in the ^{1}H-nmr spectrum of celangulin is evidence that the benzoate group is either on C-1 or C-9.

Hrms revealed that the *m/z* 202 ion was $C_{13}H_{14}O_2$. The high degree of unsaturation suggested that it contained the benzoate ester and a six-carbon fragment. It is not easy to visualize the way in which this six-carbon fragment could arise from **2** (benzoate on C-1), but a reasonable fragmentation pathway is available from **1** (benzoate on C-9). It is depicted in Scheme 1, leading to an *m/z* 202 ion in which the cation is stabilized by the adjacent oxygen atom, and the radical is tertiary and allylic. Based on the evidence presented above, **1** is proposed for the structure of celangulin.



SCHEME 1

EXPERIMENTAL[2]

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- (300 MHz) and ^{13}C-nmr (75 MHz) spectra were recorded on a GE QE-300 nmr spectrometer with TMS as internal standard and $CDCl_3$ as solvent. Uv spectrum in EtOH was obtained on a Perkin-Elmer 559 UV-VIS spectrophotometer. Eims were obtained on a Hewlett-Packard Model 5995 mass spectrometer using a direct insertion probe. Cims were obtained on a Finnigan Mat Model 4510 mass spectrometer with NH_3 or ND_3 as reagent gas. High resolution eims was obtained on a VG ZAB-2F mass spectrometer operating at 70 eV ionizing energy, 200 μA emission current, and a source temperature of 220°. The data were obtained at 10,000 resolution in the peak matching mode. Perfluorokerosene was used as the reference standard. Hplc was carried out on a Waters liquid chromatograph which was an assembly of an M-6000A pump, a U6K injector, and a Model 441 absorbance detector at 229 nm. Whatman MK6F plates were used for tlc with 1,2-dichloroethane (DCE)-methyl *t*-butyl ether (MTBE) (80:20, v/v) as solvent.

Isolation was guided by fall armyworm bioassay. It was performed with fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Noctuidae, Lepidoptera) larvae as described by Redfern (10). First instar larvae were placed on growing media containing test samples, and body weight was determined after 7 days. At a concentration in the diet of 5 ppm, the average body weight of larvae was reduced to 61%, at 10 ppm it was reduced to 37%, of the average body weight of larvae on an untreated diet. Details of biological activity will be published elsewhere.

EXTRACTION AND ISOLATION.—Dried and pulverized root bark (394 g) of *C. angulatus* (collected in Shaanxi Province, China, and authenticated by the faculty of the Department of Plant Production, Northwestern College of Agriculture, Wugon, Shaanxi, China) was extracted in a Soxhlet extractor for 24 h with Et_2O. Removal of solvent at 25° and 15 mm pressure left a crude extract as a reddish-brown semisolid (29.8 g). A portion (26.0 g) of the crude extract was covered with a mixture (500 ml) of hexane and MTBE (40:60), left standing for 24 h with occasional swirling, and decanted, and the solution was concentrated to give a reddish-brown, semisolid residue (18.4 g). This residue was chromatographed on BioSil HA® (minus 325 mesh, BIO-RAD Laboratories) (415 g, 47 × 5-cm i.d. column), and six fractions were collected: fraction 1 (hexane-MTBE, 40:60; 2 liters, 9.21 g); fractions 2 to 4 (hexane-MTBE, 20:80; 700 ml each, 2.19 g, 0.87 g, 0.54 g, respectively); and fractions 5 and 6 (MTBE; 700 ml, 0.35 g, 0.50 g, respectively). Fractions 3 and 4, in which celangulin was detected by tlc, were combined and subjected to flash chromatography on Woelm® 40 μm Si gel (116 g, 15.2 cm × 45-mm i.d. column, 50-ml fractions) first with DCE-MTBE (90:10) as the eluent (fractions 1–9), then with DCE-MTBE (85:15) (fractions 10–29). Celangulin appeared in fractions 16–21. Fraction 19 (42 mg), which contained the largest amount of celangulin, was purified by semipreparative hplc on a column (15 × 1 cm i.d.) of Spherisorb® S-10 ODS, 10 μm, with MeOH-H_2O (60:40) (flow rate 4 ml/min) as eluting solvent. Collection was made between 12.2 and 14.2 min of the peak that appeared at 12.95 min.

CELANGULIN [**1**].—Celangulin was obtained as a white amorphous powder. Uv λ max nm (ε) 231 (1800; based on molecular weight of 648), 274 (180), 281 (160). Nmr values are tabulated in Tables 1 and 2; eims *m/z* (rel. int.) 589 (0.9), 588 (2.3), 321 (1.2), 261 (2.3), 244 (5.4), 223 (3.3), 216 (3.1), 206 (4.5), 205 (3.8), 203 (7.5), 202 (35.1), 187 (3.0), 175 (3.6), 165 (5.1), 164 (9.6), 163 (6.2), 162 (3.6), 151 (4.3), 149 (4.1), 148 (3.3), 147 (3.7), 143 (3.5), 141 (4.1), 137 (3.7), 109 (3.1), 106 (8.1), 105 (100), 95 (6.0), 85 (3.6), 83 (4.3), 77 (15.2), 43 (43). Low resolution cims *m/z* 666 $[M + H + NH_3]^+$ or 671 $[M - d_1 + D + ND_3]^+$. High resolution eims *m/z* 588.2213 (calcd for $C_{30}H_{36}O_{12}$, 588.2196) and 202.0989 (calcd for $C_{13}H_{14}O_2$, 202.0990).

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LITERATURE CITED

1. M. Jacobson, "Insecticides from Plants. A Review of the Literature, 1941–1953." Agricultural Handbook No. 154, USDA, U.S. Government Printing Office, Washington, DC, 1958, p. 44.
2. Q.Q. Zhong, *Agric. Res. (Taipei)*, **1**, 29 (1950).
3. "Study and Control of Insect Pests," *Misc. Publ. Agric. Res. Bur. China*, no. 5 (Rep. 1935), 27 (1936).
4. S.F. Chiu, *J. Sci. Food Agric.*, **9**, 276 (1950).
5. W.J. Wu and G.J. Cao, *Acta Phytophylacica Sin.*, **12**, 57 (1985).

[2]Mention of proprietary names does not imply endorsement by USDA.

6. G.T. Bottger and M. Jacobson, "Preliminary Tests of Plant Materials as Insecticides," Bur. Entomol. and Plant Quart. E-796, U.S. Government Printing Office, Washington, DC, 1950.
7. R. Brüning and H. Wagner, *Phytochemistry*, **17**, 1821 (1978).
8. K. Yamada, Y. Shizuri, and Y. Hirata, *Tetrahedron*, **34**, 1915 (1978).
9. F. Delle Monache, G.B. Marini Bettolo, and E.A. Bernays, *Z. Angew. Entomol.*, **97**, 406 (1984).
10. R.E. Redfern, in: "CRC Handbook of Natural Pesticides: Methods, Vol. I, Theory, Practice, and Detection." Ed. by N.B. Mandava, CRC Press, Boca Raton, FL, 1985, pp. 479-488.
11. D.F. Hunt and S.K. Sethi, *J. Am. Chem. Soc.*, **102**, 6953 (1980).
12. C.R. Smith, Jr., R.W. Miller, D. Weisleder, W.K. Rohwedder, N. Eickman, and J. Clardy, *J. Org. Chem.*, **41**, 3264 (1976).
13. H.J. den Hertog, Jr., J.Th. Hackmann, D.D. Nanavati, and Sukh Dev, *Tetrahedron Lett.*, 845 (1973).
14. H.J. den Hertog, Jr., D. Kruk, D.D. Nanavati, and Sukh Dev, *Tetrahedron Lett.*, 2219 (1974).
15. H. Wagner, E. Heckel, and J. Sonnenbichler, *Tetrahedron Lett.*, 213 (1974).
16. H. Wagner, E. Heckel, and J. Sonnenbichler, *Tetrahedron*, **31**, 1949 (1975).
17. H. Lotter, R. Brüning, and H. Wagner, *Tetrahedron Lett.*, 3243 (1978).
18. H. Budzikiewicz and A. Römer, *Tetrahedron*, **31**, 1761 (1975).
19. H. Luftmann and G. Spitteler, *Tetrahedron*, **30**, 2577 (1974).
20. Y. Shizuri, H. Wada, K. Sugiura, K. Yamada, and Y. Hirata, *Tetrahedron*, **29**, 1773 (1973).
21. E.D. Becker, "High Resolution NMR: Theory and Chemical Applications," Academic Press, New York, 1969, p. 65.
22. S.M. Kupchan, R.M. Smith, and R.F. Bryan, *J. Am. Chem. Soc.*, **92**, 6667 (1970).
23. M. Cais, D. Ginsburg, A. Mandelbaum, and R.M. Smith, *Tetrahedron*, **31**, 2727 (1975).

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