Constituents from *Gymnema sylvestre* Leaves

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Nonacosane, hentriacontane, and tritriacontane were isolated by vapor phase chromatography from a hydrocarbon fraction of *Gymnema sylvestre* leaves. The cyclic alcohol, conduritol A, rather than the previously reported viburnitol, was also isolated from these leaves. The mass spectrum of conduritol A tetraacetate which aided in this identification is discussed.

THE ABUNDANT medical claims (1) made for crude leaf extracts of *Gymnema sylvestre* R. Br. (fam. *Asclepiadaceae*) prompted the investigation of this plant for potential medicinal agents. As an adjunct to this study, confirmation of the presence of compounds previously reported was undertaken. Because the results conflict in certain instances with earlier findings, the authors would like to report their work and offer an explanation for the disagreement in data.

EXPERIMENTAL

Compounds were first isolated from *G. sylvestre* leaves purchased from Prachi Gobeson Co., Calcutta, India, and their presence verified in the leaves of a flower-top specimen which was consistent with Hooker's (2) description of *G. sylvestre*.[1]

Melting points, unless otherwise stated, were measured on a Kofler micro hot-stage and are corrected. Microanalyses were by Spang Microanalytical Laboratories, Ann Arbor, Mich. Gas chromatographic experiments were conducted on a F&M model 720 gas chromatograph equipped with a thermal conductivity detector or model 609 equipped with a hydrogen flame ionization detector. Mass spectrometric measurements of hydrocarbons were obtained from a 90° sector magnetic scanning instrument with a heated inlet. The instrument was designed at Dow Chemical Co., Midland, Mich. The spectrum of conduritol A tetraacetate was measured with a CEC 21–103C mass spectrometer equipped with a heated (200°) all glass inlet system. The ionizing energy was maintained at 70 e.v. and the ionizing current at 50 μamp. Sixty-megacycle nuclear magnetic resonance spectra were measured with a Varian model A-60 analytical NMR spectrometer. Infrared spectra were recorded on a Perkin-Elmer model 337 infrared spectrometer.

Isolation of Hydrocarbons.—Dried, powdered leaves (12 mesh, 7.9 Kg.) were macerated with petroleum ether (30–60°) for 24 hr. The solution was drawn off and the leaves continuously extracted in a Lloyd extractor with 6 gal. of fresh solvent for 24 hr. The marc was set aside for further treatment and the petroleum ether extracts combined. Removal of solvent left 115 Gm. of a waxy, green solid which was chromatographed, in portions, over 60/80 mesh Florisil (about 20 Gm. Florisil/Gm. of extract). A white semisolid was obtained by elution with petroleum ether (30–60°). Recrystallization from acetone afforded 6.0 Gm. of a white solid, m.p. 64–65° (uncorrected).

Anal.—Found: C, 85.58; H, 14.37.

This white solid was subjected to gas chromatographic analysis under the following conditions: column, 2 ft., 20% silicone gum rubber on Chromosorb P; He flow rate, 50 ml./min.; column temperature, 270°, isothermal; injection port temperature, 310°; detector, thermal conductivity cell at 342°; solvent, petroleum ether (90–100°). Five components were revealed exhibiting retention times (R_t) of 3.2, 4.0, 5.2, 6.5, and 8 min., respectively. Corresponding area ratios were 12.8:0.01:26.8:02:10. Major constituents were collected. A check of separation efficiency by rechromatography using the same procedure but with cyclohexane as solvent and a flame ionization detector showed only one peak for samples of R_t 3.2 and 5.2. However, the last compound (R_t 8) contained 1–2% of another component eluted before it. Physical data for the isolated hydrocarbons in the order of compound, molecular weight by mass spectrum, R_t, melting point found [reported (3)] are as follows: nonacosane (*A*), 408, 3.2 min., 63.5–63.7° (63.7°); hentriacontane (*C*), 436, 5.2 min., 67.7° (67.9°); tritriacontane (*E*), 464, 8 min., 71.5–71.8° (71.4°).

Isolation of Conduritol A (Compound I).—The marc from the petroleum ether extraction described above was extracted continuously with methanol for 4 days. At approximately 8-hr. intervals, methanol extract was removed and replaced with an equal quantity of fresh solvent. Ultimately, all extracts were combined. Removal of solvent under reduced pressure left a viscous oil which was taken up in water. Water-insoluble acids were precipitated at pH 2 and bases were then removed by chloroform extraction of the mother liquor after adjustment to a basic pH. The aqueous solution following this extraction was neutralized and diluted to 5 times its volume with acetone, whereupon a red-brown oil deposited. The supernatant solution was freed of solvent and the yellow-oil residue taken up in boiling ethanol and filtered. Conduritol A crystallized from this solution on cooling.

Conduritol A.—The analytical sample was prepared by 5 recrystallizations from ethanol to give stout rods, m.p. 145.5–145.7°; lit. (4) m.p. 142–143°. NMR in deuterium oxide with external tetramethylsilane standard, 6.02 δ (2H, doublet, *J* 1 c.p.s., vinyl protons), 4.40 δ (2H, quartet, *J* 3.5 and 1 c.p.s.,

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[1] The authors are grateful to Dr. A. G. Paul for this comparison of the flowering top material purchased from The Himalaya Drug Co., Bombay, India.

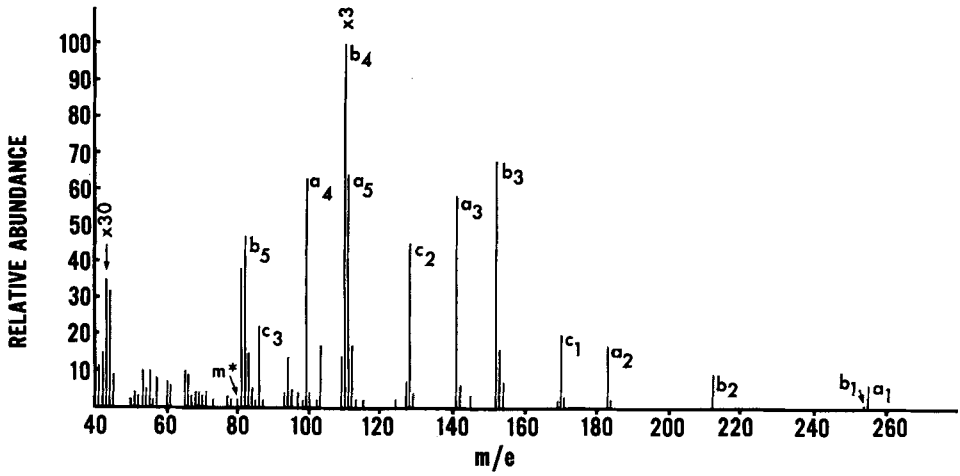


Fig. 1.—Mass spectrum of conduritol A tetraacetate.

CHOH) and 4.02 δ (2H, doublet, J 3.5 c.p.s., C=C CHOH).

Anal.—Calcd. for $C_6H_{10}O_4$: C, 49.31; H, 6.89. Found: C, 49.38; H, 7.06.

Periodate oxidation was performed by adding 42 ml. of water, 0.3 ml. of 1 N sulfuric acid, 15 ml. of 0.03 M sodium metaperiodate, and 3 ml. of 0.0075 M conduritol A to a glass-stoppered flask placed in a bath at 25 ± 0.1°. The solution was sampled at appropriate times, the number of moles of periodate used per mole of compound determined by the Fleury-Lange method (5), and the following results recorded: 5 min., 2.49; 30 min., 3.04; 23 hr., 3.11.

Conduritol A Tetraacetate.—Acetylation of 0.395 Gm. (27 mmoles) of conduritol A was conducted with 3 ml. of acetic anhydride, 0.2 Gm. of sodium acetate, and heating on a steam cone for 2 hr. After hydrolysis of excess acetic anhydride in ice water, the acetate was extracted into ether. The ether solution was dried and solvent removed. The crude sample was chromatographed from neutral alumina with benzene followed by ether to give 0.80 Gm. (95%) of a viscous oil, b.p. 130° at 0.05 mm. A sample for analysis was rechromatographed from alumina. NMR in deuterochloroform with tetramethylsilane internal standard, 5.95 δ (2H, doublet, J 1.5 c.p.s., vinyl protons), 5.44 δ (4H, poorly resolved doublet, CHOAc) and 2.1 δ (12H, doublet, J 1.5 c.p.s., methyl protons); mass spectrum (Fig. 1) m/e 255 (ion a_1), 254 (ion b_1), 212 (ion b_2), 183 (ion a_2), 170 (ion c_1), 152 (ion b_3), 141 (ion a_3), 128 (ion c_2), 111 (ion a_5), 110 (ion b_4), 99 (ion a_4), 86 (ion c_3), and 82 (ion b_5).

Anal.—Calcd. for $C_{14}H_{18}O_8$: C, 53.50; H, 5.77; sapon. equiv., 312. Found: C, 53.44; H, 5.97; sapon. equiv., 321.

Dihydroconduritol A.—Methanol (15 ml.) was added to 0.1993 Gm. (1.37 mmoles) of conduritol A and the solution hydrogenated over platinum oxide at atmospheric pressure and 30°. Hydrogen uptake stopped at 1.29 moles/mole of sample. The catalyst was filtered off and solvent removed under reduced pressure leaving 0.179 Gm. (90%) of residue, m.p. 166–170°. Recrystallization from acetone–chloroform yielded a compound, m.p. 198–203°; lit. m.p. 198° (6), 204° (7), and 210° (8, 9).

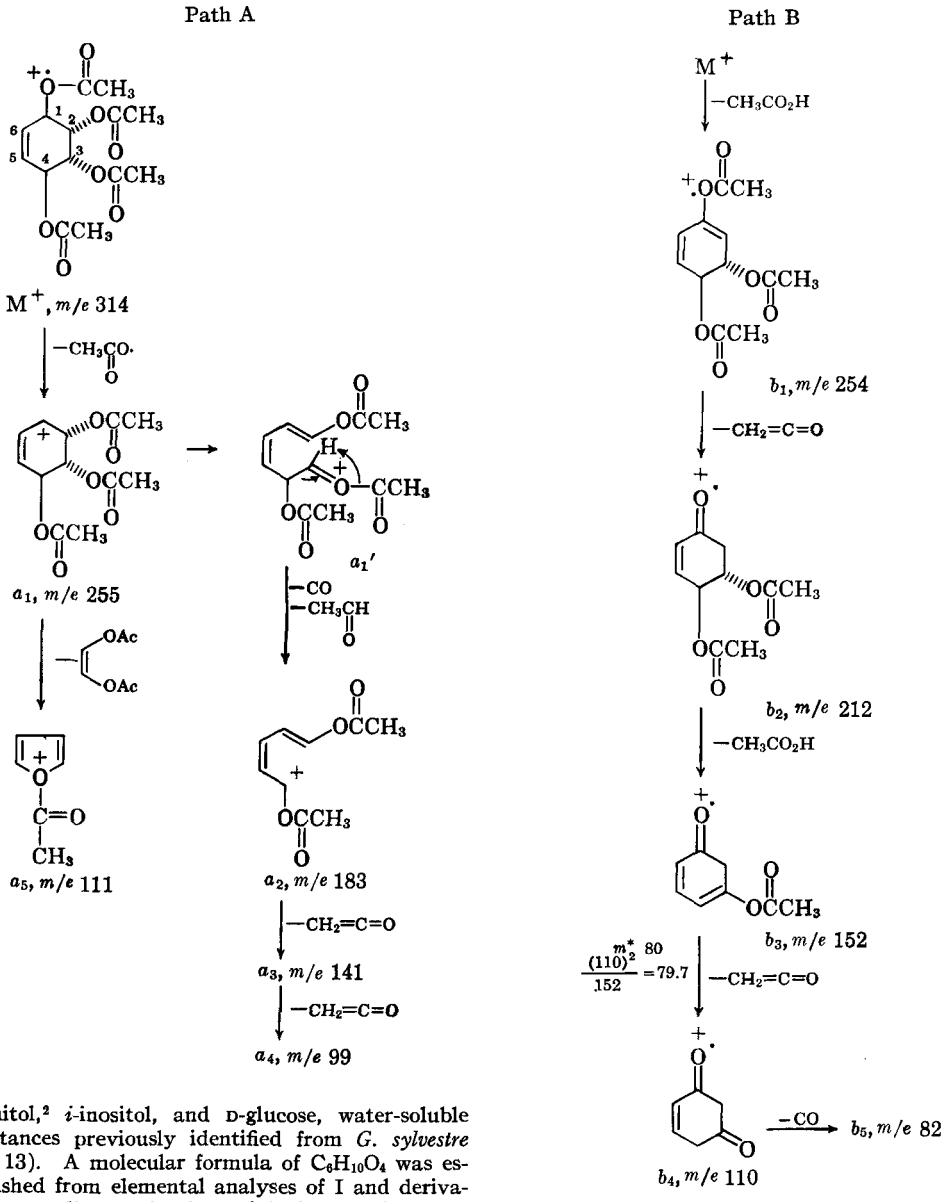
Anal.—Calcd. for $C_6H_{12}O_4$: C, 48.64; H, 8.16. Found: C, 48.94; H, 8.29.

RESULTS AND DISCUSSION

Petroleum ether extraction of powdered leaves removed a solid from which a hydrocarbon fraction (0.08% of dried plant), m.p. 64–65°, was isolated by chromatography over Florisil and recrystallization from acetone. Solid-state infrared spectra of the fraction showed a doublet from 719–730 cm.$^{-1}$ which, for solid alkanes, suggests either a chain length of 26 or more carbons or an odd-carbon length of less than 26 (10). Melting point data support the former assignment.

Vapor phase chromatography of the hydrocarbon fraction revealed the presence of 5 components in the ratio of 12.8:0.01:26.8:0.02:10. The major constituents (labeled A, C, and E in the order of their elution) were collected and submitted to mass spectrometric analysis. Molecular ions of 408, 436, and 464 were observed for A, C, and E, respectively. Furthermore, the fragmentation pattern of each compound exhibited an initial loss of 29 mass units with other fragments appearing every 14 mass units below this. Peak intensities gradually increased to a maximum at m/e 57. These features, described as characteristic for n-alkanes (11), show that substances A, C, and E are nonacosane, hentriacontane, and tritriacontane, respectively. Melting points of chromatographically purified samples are in excellent agreement with published values and reinforce the above conclusions. Hentriacontane has been isolated from *G. sylvestre* by Power and Tutin (12). In addition, Mhaskar and Caius (13) indicated the presence of pentatriacontane; vapor phase chromatographic examination of hydrocarbon fractions from several samples of dried plant has failed to reveal this compound.

While screening for alkaloids, a water-soluble optically inactive alcohol (I), m.p. 145.5–145.7°, was isolated and easily differentiated from vi-



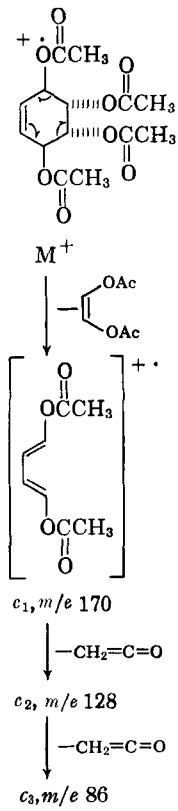
burnitol,[2] *i*-inositol, and D-glucose, water-soluble substances previously identified from *G. sylvestre* (12, 13). A molecular formula of $C_6H_{10}O_4$ was established from elemental analyses of I and derivatives as well as molecular weight information from the saponification equivalent and mass spectrum of its tetraacetate (*vide infra*). Catalytic hydrogenation of the alcohol yielded a dihydro derivative accompanied by the uptake of 1.29 moles of hydrogen. Quantitative periodic acid oxidation of I resulted in 3.11 moles of periodate reacting per mole of compound. The above data suggest that I possesses one double bond, one ring, and four vicinal hydroxyl groups; confirmation is derived from an examination of NMR spectra. In deuterium oxide, the spectrum of I shows three sets of protons. A pair of vinylic protons appears as a doublet (*J* 1 c.p.s.) at 6.02 δ. The remaining four protons are equally divided between a quartet centered at 4.40 δ (*J* 3.5 and 1 c.p.s.) and a doublet at 4.02 δ (*J* 3.5 c.p.s.); the position of this group indicates that they are on carbon attached to oxygen. The tetraacetate of I shows three sets of peaks, including a CH_3 resonance at 2.1 δ, in a ratio of 1:2:6.5. As expected, the >CHOAc protons are deshielded and so appear downfield relative to the parent alcohol (15).

These chemical and physical properties are consistent with those which would be exhibited by the *meso* (1,4/2,3) diastereomer of 1,2,3,4-cyclohexanetetrol (conduritol A) isolated by Kubler from *Marsdenia condurango* another member of the family *Asclepiadaceae* (4). Ultimately, comparison with an authentic sample[3] showed I and conduritol A to

[2] When first isolated (12) the compound was referred to as an *l*-quercitol. Only when its configuration was established (14), did its identity with viburnitol become apparent. The authors are grateful to the Wellcome Laboratories for Tropical Medicine, London, England, who supplied a reference sample.

[3] The authors are indebted to T. Posternak, University of Geneva, Switzerland, for this sample.

Path C



be identical. Also, catalytic hydrogenation yielded identical dihydro derivatives.

An extensive examination of *G. sylvestre* leaves failed to show the presence of viburnitol. Indeed, when we duplicated the isolation procedure of Power and Tutin (12) for viburnitol, only conduritol A was obtained. Since no doubt exists as to the structure of either compound, the explanation may lie in the identity of the plant. Power and Tutin (12) mention neither the source of their leaves nor any attempt to compare them with herbarium specimens. Hooker (2) indicates that except for a detailed comparison of the flowering plants it is questionable whether *G. hirsutum* can be distinguished from *G. sylvestre*. This problem in identification is especially significant because a simple device to distinguish *G. sylvestre* from many related species is to chew the leaves and observe a loss of ability to taste sugar. However, *G. hirsutum* and, to a lesser extent *G. montanum* are also reported (16) to contain gymnemic acid, the fraction responsible for this effect.

The molecular weight determination of conduritol A tetraacetate by mass spectrometry has been mentioned. The basis for this assignment and a brief discussion of the genesis of major peaks in the spectrum follows.

Fragmentation processes involved in the mass spectrum (Fig. 1) of conduritol A tetraacetate generally resemble those previously described for peracetylated carbohydrates; *i.e.*, loss of acetic acid and/or ketene represent the prevalent modes of fragmentation (17). Three pathways (A–C) can be formulated to account for the majority of peaks.

Path A proceeds *via* loss of the acetoxy function at C-1 or C-4 producing an allylically stabilized ion at m/e 255 (a_1). The analogous cleavage in hexose and pentose polyacetates is the loss of acetoxy from C-1, α to the ring oxygen thereby deriving stabilization from the free electron pair on oxygen (17). Although no molecular ion is visible, the appearance of a_1 as the characteristic peak of largest mass is presumptive evidence for a molecular weight of 314. Decomposition of a_1 may occur in two ways. The first is expulsion of carbon monoxide and acetaldehyde from a valence isomer of a_1 (a_1') giving a_2 (m/e 183). Successive elimination of ketene then accounts for species a_3 (m/e 141) and a_4 (m/e 99). On the other hand, retro-Diels-Alder collapse of a_1 generates the peak at m/e 111 (a_5).

A second series of ions (path B) is produced from the molecular ion (M^+) by the alternate loss of acetic acid and ketene, yielding fragments b_1 (m/e 254), b_2 (m/e 212), b_3 (m/e 152) and b_4 (m/e 110). The m/e 254 peak is extremely weak, amounting to only 0.5 units on the scale used in Fig. 1, most likely indicating the large driving force for production of b_2. A metastable peak at m/e 80 confirms the transformation $b_3 \rightarrow b_4$. Assignment of b_4 as a diketone tautomeric with resorcinol is consistent with both the intensity of m/e 110 and the presence of an ion 28 mass units lower (m/e 82), for the spectrum of resorcinol itself exhibits a pronounced molecular ion as well as an M-28 peak ascribed to the loss of carbon monoxide (18). Other mechanisms may be advanced for the production of fragments b_2 through b_4. However, in the absence of labeling experiments, it is not profitable to discuss them here.

Retro-Diels-Alder collapse of M^+ initiates path C. The ion formed (c_1 = m/e 170) then loses 2 moles of ketene giving rise to c_2 (m/e 128) and c_3 (m/e 86). The process $c_1 \rightarrow c_2 \rightarrow c_3$ has been recognized in the spectrum of β-D-2-deoxyglucopyranose tetraacetate and has been confirmed by deuterium labeling (17).

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