A Novel Cardenolide, Canarigenin-3-O-#-L-rhamnopyranosyl-(1#5)-O-#-D-xylofuranoside, from Rhizomes of Convallaria majalis

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A NOVEL CARDENOLIDE, CANARIGENIN-3-*O*-α-L-RHAMNOPYRANOSYL-(1↦5)-*O*-β-D-XYLOFURANOSIDE, FROM RHIZOMES OF *CONVALLARIA MAJALIS*

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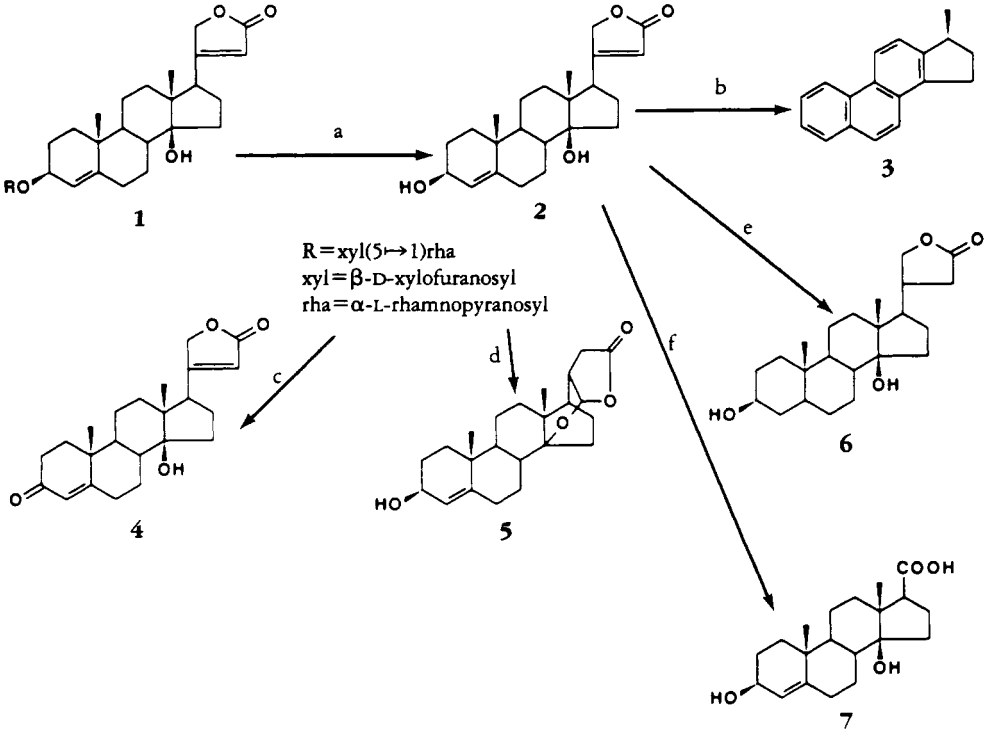
ABSTRACT.—A novel cardenolide, canarigenin-3-*O*-α-L-rhamnopyranosyl-(1↦5)-*O*-β-D-xylofuranoside [**1**], has been isolated from an EtOAc-soluble fraction of the rhizomes of *Convallaria majalis* and identified by chemical and spectral analysis.

Convallaria majalis L. (Liliaceae) (1) is reputed to have therapeutic value. It is found throughout the plains of India. Earlier workers (2) have reported the presence of cardiac constituents in *Convallaria* species.

RESULTS AND DISCUSSION

The EtOAc-soluble part of the EtOH extract of rhizomes of *C. majalis* when worked up gave compound **1** (0.086%), mp 174–176°, molecular formula $C_{34}H_{50}O_{12}$, $[M]^+$ 650, and $[\alpha]^{27}D$ +127 (pyridine). It gave a positive Kedde's reaction (3), a positive Keller-Kiliani reaction (4) and a positive Legal test (5), indicating its cardenolide nature.

Acid hydrolysis (7% ethanolic H_2SO_4) of **1** gave compound **2** (canarigenin) and sugar moieties (Scheme 1). Compound **2**, mp 233–235°, analyzed for $C_{23}H_{32}O_4$; $[M]^+$



SCHEME 1. a) 7% ethanolic H_2SO_4; b) selenium at 320°; c) CrO_3/pyridine; d) KOH; e) catalytic hydrogenation; f) acetylation, $KMnO_4$ in Me_2CO, hydrolysis.

372 and $[\alpha]^{27}D$ +31.5 (in $CHCl_3$). It gave Diel's hydrocarbon [**3**] on dehydrogenation with selenium at 320°, molecular formula $C_{18}H_{16}$, mp 126–128°.

A peak in the ir spectrum of **2** at 3596 cm^{-1} indicated OH group(s). The presence of OH group(s) was confirmed by ^1H nmr of the monoacetyl derivative of **2**. The ^1H nmr of **2** gave singlets at δ 2.18 for OAc and δ 3.54 for OH. Acetylation of **2** formed a monoacetate $C_{25}H_{34}O_5$, mp 190–192°, thereby confirming the presence of two OH group(s) in **2**. One OH group was either primary or secondary, and the other OH was tertiary.

CrO_3/pyridine oxidation of compound **2** yielded a ketone **4**, $C_{23}H_{30}O_4$, mp 198°, $[M]^+$ 370, which showed a positive Zimmermann test (6) for the C-3 keto group, thereby confirming the presence of one -OH group at C-3 and further indicating its nature as secondary. Compound **2**, on reaction with KOH, formed isocardiogenin [**5**] (7), $C_{23}H_{32}O_4$, mp 270°, $[M]^+$ 372. Formation of compound **5** was explained by the presence of the tertiary OH group at C-14 (8).

Other peaks at ν max (KBr) 1785 cm^{-1} and 1735 cm^{-1} indicated the presence of a lactone ring in compound **2**. The position of the lactone ring in compound **2** was established at C-17 since the monoacetate on oxidation with $KMnO_4$ in Me_2CO followed by hydrolysis yielded a compound, molecular formula $C_{20}H_{30}O_4$, which was found to be 3β,14β-dihydroxy-4,5-dehydroetianic acid (9) (confirmed by mmp with an authentic sample).

A peak in the ir spectrum at ν max (KBr) 1640 cm^{-1} showed the presence of unsaturation which was further supported by the fact that compound **2** on catalytic hydrogenation formed a tetrahydrocardiogenin **6**, $C_{23}H_{36}O_4$, mp 202°, $[M]^+$ 376, thereby confirming the presence of two double bonds. The position of one double bond was fixed in the steroidal nucleus, since compound **2** gave a positive TNM test, thereby ruling out the possibility of a double bond in the steroidal nucleus. This was confirmed by oxidation of the monoacetate with $KMnO_4$ in Me_2CO, followed by hydrolysis, which yielded 3β,14β-dihydroxy-4,5-dehydroetianic acid [**7**], $C_{20}H_{30}O_4$, $[M]^+$ 334. Thus, the position of one double bond in compound **2** is at C-4.

When compound **2** was treated with $Na_2[Fe(NO)(CN)_5]$ in C_5H_5N, a deep red color was produced, which indicated the presence of an α,β-unsaturated lactone ring. This confirmed a double bond at C-20(22) (10) and suggested that compound **2** was 4,5-anhydroperiplogenin (canarigenin).

Attachment of sugars to the cardiogenin in the glycoside was fixed at C-3, as the glycoside itself did not give a positive Zimmermann test. The cardiogenin did test positive, thereby confirming that the 3-OH group was free in the cardiogenin but was bonded in the glycoside.

On partial hydrolysis with 2% H_2SO_4, **1** first showed L-rhamnose (11) followed by D-xylose, confirming that L-rhamnose was the terminal sugar and that D-xylose was attached to the cardiogenin. Hydrolysis of **1** by the enzyme in Taka-Diastase® yielded L-rhamnose. On further hydrolysis by the enzyme almond emulsin, D-xylose was obtained (12). On oxidation with HIO_4, compound **1** consumed 3.04 mol of periodate (13) to produce 1.03 mol of HCO_2H per mol of **1**, thereby indicating that rhamnose was in the pyranose form and that xylose was in the furanose form. This was further supported by acid hydrolysis of permethylated **1**, which yielded 2,3-di-*O*-methyl-D-xylose (confirmed by co-tlc) and 2,3,4-tri-*O*-methyl-L-rhamnose (confirmed by co-tlc). Thus, the identity of compound **1** is canarigenin-3-*O*-α-L-rhamnopyranosyl-(1→5)-*O*-β-D-xylofuranoside.

EXPERIMENTAL

PLANT MATERIAL.—*C. majalis* was procured from M/s. United Chemical and Allied Products, Cal-

cutta. It was authenticated by the Department of Botany, courtesy of Dr. Harisingh, Gour University, Sagar, India. A herbarium specimen (No. V-XVII) has been deposited in the Chemistry Department of this university. Powdered rhizomes from *C. majalis* (3 kg) were extracted with 90% EtOH. The extract was concentrated under reduced pressure to a viscous mass and was then segregated into petroleum ether- (60–80°), C_6H_6-, $CHCl_3$-, EtOAc-, and Me_2CO-soluble fractions.

ISOLATION.—The concentrated EtOAc-soluble extract showed three spots on tlc [Si gel G, EtOAc-MeOH-H_2O (12:1:1), I_2 vapor visualization]. The extract was purified over a Si gel column (60–120 mesh) and eluted with $CHCl_3$/EtOAc. Eluates from fractions 1–13 and 20–26 had the same R_f value, and fractions 14–19 provided compound **1** (0.086%): colorless, microcrystalline solid; mp 176–178°, $C_{34}H_{50}O_{12}$ (found C 62.69, H 7.67; calcd C 62.72, H 7.69), eims $[M]^+$ 650; uv λ max (MeOH) 217, 290 nm; ir ν max (KBr) 3596, 2965, 1785–1735, 1640, 1460, 1390, 1350, 1315, 1260, 1190, 1070 cm^{-1}; ^{1}H-nmr (90 MHz, DMSO) of pentaacetyl derivative of compound **1** 0.68 (s, 3H, Me-18), 0.96 (s, 3H, Me-19), 5.86 (d, J = 7 Hz, 1H, H-4), 1.3–2.0 (polymethylene CH_2 and CH), 4.86 (s, 2H, H_2-21), 4.48 (m, 1H, H-3), 3.56 (s, 1H, 14-OH), 2.84 (m, 1H, H-17), 5.82 (s, 1H, H-22), 5.51 (d, J = 7 Hz, 1 anomeric proton, H-1′), 5.54 (d, J = 7 Hz, 1 anomeric proton, H-1″), 3.60–4.40 (m, 9 sugar protons), 2.15 (s, 3H, 2′-OAc), 2.17 (s, 3H, 3′-OAc), 2.13 (s, 6H, 2″- and 3″-OAc), 2.07 (s, 3H, 4″-OAc), 1.10 (d, J = 6 Hz, Me-6″); ^{13}C nmr (20.0 MHz, DMSO, ppm), 27.8 (C-1), 28.26 (C-2), 73.82 (C-3), 116.88 (C-4), 138.88 (C-5), 28.89 (C-6), 26.89 (C-7), 32.28 (C-8), 48.86 (C-9), 58.35 (C-10), 21.39 (C-11), 31.88 (C-12), 49.38 (C-13), 83.39 (C-14), 28.85 (C-15), 28.81 (C-16), 48.86 (C-17), 15.64 (C-18), 14.86 (C-19), 176.37 (C-20), 73.36 (C-21), 126.69 (C-22), 172.89 (C-23), 102.68 (C-1′), 73.38 (C-2′), 76.86 (C-3′), 77.89 (C-4′), 63.89 (C-5′), 101.89 (C-1″), 72.86 (C-2″), 76.68 (C-3″), 70.84 (C-4″), 74.88 (C-5″), 49.38 (C-6″); eims *m/z* (%) $[M]^+$ 650 (25), 504 (15), 372 (31), 354 (17), 338 (18), 201 (68), 160 (11), 145 (19).

ACID HYDROLYSIS.—Compound **1** was hydrolyzed (7% ethanolic H_2SO_4) by refluxing for 8 h and yielded compound **2**. The hydrolysate was neutralized with $BaCO_3$, $BaSO_4$ was filtered off, and the filtrate was concentrated under vacuum. The residue was examined by tlc [*n*-BuOH–HOAc–H_2O (4:1:5)] and showed the presence of L-rhamnose and D-xylose (12).

IDENTIFICATION OF THE CARDIOGENIN **2**.—Colorless microcrystalline solid; mp 233–235°, $C_{23}H_{32}O_4$ (found C 74.16, H 8.56; calcd C 74.19, H 8.60); eims $[M]^+$ 372; tlc homogeneous [R_f 0.62, EtOAc-MeOH-H_2O (12:1:1)]; $[\alpha]^{27}D$ +31.5 (in $CHCl_3$); uv λ max (MeOH) 218, 292 nm, ir ν max (KBr) 3560, 2965, 1780–1730, 1650, 1445, 1375, 1345, 1310, 1255, 1185, 1080 cm^{-1}; ^{1}H nmr (90 MHz, DMSO) of monoacetyl derivative of **2** 0.70 (s, 3H, Me-18), 0.94 (s, 3H, Me-19), 5.86 (d, J = 7 Hz, 1H, H-4), 1.2–2.0 (polymethylene CH_2 and CH), 4.82 (s, 2H, H_2-21), 4.44 (m, 1H, H-3), 3.54 (s, 1H, 14-OH), 2.84 (m, 1H, H-17), 5.80 (s, 1H, H-22); ^{13}C nmr (20.0 MHz, DMSO, ppm) of **2** 27.6 (C-1), 28.46 (C-2), 73.66 (C-3), 116.76 (C-4), 138.72 (C-5), 28.75 (C-6), 26.78 (C-7), 32.36 (C-8), 48.94 (C-9), 58.44 (C-10), 21.46 (C-11), 31.72 (C-12), 49.44 (C-13), 83.48 (C-14), 28.56 (C-15), 28.56 (C-16), 49.12 (C-17), 15.76 (C-18), 14.80 (C-19), 176.24 (C-20), 73.76 (C-21), 126.54 (C-22), 172.74 (C-23); eims *m/z* (%) $[M]^+$ 372 (31), 354 (17), 338 (18), 201 (68), 160 (11), 145 (19).

PERIODATE OXIDATION.—Compound **1** was dissolved in MeOH and treated wtih $NaIO_4$ for 2 days. The liberated HCOOH and consumed periodate were estimated by the Jones method (13).

ENZYMATIC HYDROLYSIS.—Compound **1** in EtOH was treated with an equal volume of Taka-Diastase® and almond emulsion solution and left at room temperature for 24 h. Examination of the hydrolysate on tlc showed the presence of L-rhamnose and D-xylose.

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LITERATURE CITED

1. R.N. Chopra, S.L. Nayar, and B.S. Verma, "Supplement to Glossary of Indian Medicinal Plants," CSIR Publication, New Delhi, 1968, p. 19.
2. K.K. Chen, A.I. Chen, and R.C. Anderson, *J. Am. Pharm. Assoc.*, **25**, 539 (1936).
3. J.A. Hembree, C.J. Chang, J.L. McLaughlin, G. Peck, and J.M. Cassady, *J. Nat. Prod.*, **42**, 293 (1979).
4. J.V. Euw and T. Reichstein, *Helv. Chim. Acta*, **31**, 883 (1948).
5. M. Keller and T. Reichstein, *Helv. Chim. Acta*, **32**, 1607 (1949).
6. D.H.R. Barton and R.D. Mayo, *J. Chem. Soc.*, 887 (1954).
7. W.A. Jacobs and E.L. Gustus, *J. Biol. Chem.*, **74**, 811 (1927).
8. M.P. Khare, O. Schindler, and T. Reichstein, *Helv. Chim. Acta*, **45**, 1536 (1962).

9. S. Smith, *J. Chem. Soc.*, 2478 (1930).
10. W.A. Jacobs and A. Hofmann, *J. Biol. Chem.*, **79**, 519 (1928).
11. F.G. Mann and B.C. Saunder, "Practical Organic Chemistry," Longman, New York, 1936, p. 1369.
12. S.P. Mishra and V.K. Mohan Rao, *J. Sci. Ind. Res. Sect. C*, **19**, 170 (1960).
13. E.L. Hirst and J.K.N. Jones, *J. Chem. Soc.*, 1959 (1949).

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