TRACE GLYCOSIDE FROM CRANBERRIES (*VACCINIUM OXYCOCCUS*)

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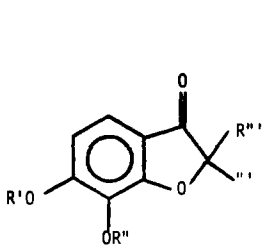
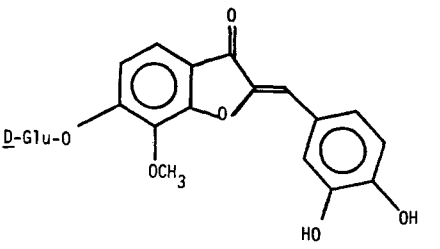
ABSTRACT.—A glycoside extracted from cranberries (*Vaccinium Oxycoccus* L.) was found to have the structural characteristics of leptosine **2**. The structure was proven by various spectroscopic methods and by direct comparison with authentic material. The presence of the unusual moeity **1** in leptosine and in other minor alkaloids from the cranberries raises several questions regarding their biosynthetic precursors and their role in biosynthesis.

We reported earlier the presence of two new intriguing families of alkaloids from cranberry extracts (*Vaccinium Oxycoccus* L.) (1,2). The interest in these specimens lay mainly in their use by various natives (Eastern Europe) as a popular medicine said to possess anticancer or antifebral activity. The mass spectra study of minor alkaloids showed the presence of a highly unusual aromatic moeity having partial structure **1** (3–5). We have further concentrated on the phenolic (weakly acidic) fraction of the freeze-dried total methanolic extract (1,2), where we could find strong evidence of the presence of pigments (e.g. antochlor type, thus rendering the extract more interesting).

To our surprise, a highly polar component, thought to be a glycoside, exhibited in its mass spectrum a fragmentation pattern characteristic to moeity **1**. Further spectroscopic analyses led to the assignment of structure **2** for that substance. Compound **2** is known as leptosine and was reported to be found in *Coreopsis grandiflora* Nutt[1] (6–8). We obtained an authentic sample of leptosine, compared the various spectroscopic data, and came to the conclusion that our early assignment of structure **2** was correct (mp 233–7, dec. hydrate). A rigorous structure proof by means of spectroscopic data is now presented.



1



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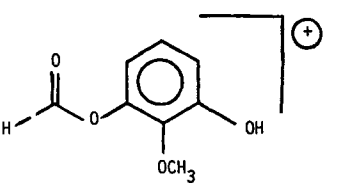

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[1]Sample of authentic leptosine obtained from Professor M. Nogradi, Technical University, Budapest, Hungary.

The mass spectrum of **2**, which exists as a dihydrate, shows a weak molecular ion at *m/z* 462 (0,4%). High resolution measurements confirmed the molecular formula as being $C_{22}H_{22}O_{11}$ (found: 462,0602 calc. 462,0560). Weaker peaks can also be seen for the dihydrate (*m/z* 498) and the monohydrate (*m/z* 480) forms. Table 1 presents the mass spectral data for **2** along with the data from authentic leptosine. The major fragmentation pathways result from the cleavage of an auron ring alpha to the carbonyl (462→300, loss of 162) from the molecular ion and cleavage of the glucose moeity at the anomeric carbon with the formation of the characteristic $H-C=\overset{+}{O}-R$ fragment at *m/z* 312 (9).

Breakdown of the *m/z* 312 leads to the loss of either CO (*m/z* 284) or HCO (*m/z* 283). The other important fragments at 167 ($C_8H_7O_4$) 166 and 138 ($C_7H_6O_3$) correspond to methoxy phenolic formate moeity **3**, **3**–H and **3**–HCO, respectively. Two other peaks at *m/z* 73 and 60 correspond to the sugar fragments $C_3H_5O_2$ and $C_2H_4O_2$. The mass spectral analysis of persilylated leptosine (TMSC, pyridine (10)) agrees very well with structure **2**.

TABLE 1. Mass spectral data for **2**.

m/z	**2** I	*Leptosine* (5,6) I
498	0.2	0.6
480	0.2	1.7
462 (M^+)	0.4	0.5
312	2.4	2.7
300	100.0	100.0
284	5.4	4.0
283	3.6	3.0
277	8.2	7.0
239	3.0	1.0
167	15.5	12.4
166	11.3	10.1
149	2.3	16.2
138	28.2	22.9
123	14.9	15.5
119	13.6	10.2
73	32.4	26.7
60	52.4	61.1
57	37.3	32.2
43	33.8	30.1

The proton nmr spectrum of **2** corresponds to the proposed structure (table 2). The methoxy protons (4,07 ppm), the four exchangeable glucose hydroxyl protons (centered at 3,35 ppm) and the anomeric proton (5,40 ppm) are all easy to assess. Two phenolic proton signals at 9,35 and 9,75 ppm have been assigned by exchange with D_2O and electronic considerations (they are *m*- or *p*- to the α,β-unsaturated system). The remaining sugar and methylene protons are centered around 7,1 ppm. The singlet for H–2' at 6,7 ppm is followed in order of increasing δ by H–5, H–5', H–6' and H–4.

The ^{13}C spectrum (table 3) provides more information about the structure of leptosine. Twenty-two carbon signals are recorded: one quadruplet, one triplet, nine singlets and eleven doublets (from off-resonance experiments). The assessment of $C=O$ (181,59 ppm), OCH_3 (60,48 ppm), C–6" (60,94 ppm) and the five other sugar moeity signals (70–100 ppm) is simple. The remaining singlets are assigned in table 3. The group of signals C–7, C–4', C–3', C–6 and C–1a is assigned by use of model compounds, all of sp^2 =C–OR type. The highest field absorption corresponds to C–6 because of the sugar substituent on this carbon. Of the next three singlets, C–3a is shifted by about 2 ppm from the corresponding peak of acetophenone (11) for instance, while C–1' shows a large shift compared

TABLE 2. ^{1}H Nmr data (δ_x, ppm).[a]

OCH_3	4.07 (s)
H-1"	5.40 ($J_{1",2"}$ 8 Hz)
H-2", 3", 4", 5"	5.1 (centre)
CH_2	3.70 (d)
H-2'	6.70 (s)
H-2a	7.53 (s)
H-4	7.40 (d)
H-5	6.85 (d)
H-5'	7.10 (d)
H-6'	7.25 (d)
OH (glucose)[b]	3.35 (centre)
OH-4'[b]	9.75
OH-3'[b]	9.35

[a]Varian XL-100 in DMSO-d_6 after the exchange with D_2O and the liophylisation (TMS at $\delta=0$).
[b]Disappeared after D_2O exchange.

to styrene because of the combined effects of para-phenol and enol-carbonyl groups. The last singlet identified as C-2 at 116,9 ppm is easily justified by its intensity; for some enols in aromatic series, both enolic carbons are almost twice as intense as the aromatic carbons.[2] The doublet for C-2a at 145,2 ppm has been assigned according to either models of the O=C–C=CH–Ø type or cinnamic acid derivatives (11). The 3,4-dihydroxycinnamic acid is quite a good model of the ^{13}C spectrum for **2**. However, the α-carbon-OR substituent has an important influence on δ_c[3].

TABLE 3. ^{13}C Nmr data for **2**.[a]

OCH	60.48 (q)
C-6"	60.94 (t)
C-5"	73.17 (d)
C-4"	69.47 (d)
C-3"	77.19 (d)
C-2"	76.59 (d)
C-1"	100.43 (d)
C-3	181.59 (s)
C-7	148.40 (s)
C-4"	145.47 (s)
C-3'	156.41 (s)
C-3a	134.19 (s)
C-2a	145.16 (d)
C-6	157.32 (s)
C-1a	113.01 (s)
C-1'	124.79 (s)
C-2	116.97 (s)
C-4	118.64* (d)
C-2'	117.99* (d)
C-6'	123.07 (d)
C-5'	215.98* (d)
C-5	112.04* (d)

[a]Varian FT80A in DMSO-d_6 δ_x (TMS at $\delta=0,00$).
*These assignments may be interconverted.

[2]K. Jankowski, unpublished study of enols.
[3]Carbon-13 spectrum of 3,4-dihydroxycinnamic acid in DMSO-d_6 shows peaks at 168,3 (C=O), 144,7 and 122,9 (C=C), 148,1 (C-4), 148,7 (C-3), 125,0 (C-1), 115,7, 115,9 and 116,2 (other aromatic carbons).

The assignment of the remaining five aromatic carbons was quite difficult as all of them are p- or o- to OH (or OR) groups. Logically carbons C–4, C–2' and C–6' could be placed at lower field than C–5' and C–5 The carbon C–4 has been tentatively assigned the peak at 118,6 ppm. The difference between C–2' and C–4 could be due to the C–3' hydroxyl. However the last five carbon assignments may be interconverted.

This work definitely proves the structure of leptosine, **2**. In addition, however, it raises a series of questions regarding the nature of the basic skeleton, i.e., moeity **1**. In fact, to date, this moeity has not been shown to be part of any important biosynthetic pathway. Yet its presence in two different types of plant material and especially its presence in alkaloids (basic fraction) and in a "phenolic glycoside" (weakly acidic fraction) suggests an important biosynthetic role. Alkaloids and glycosides are not present in the same cells in a plant specimen. This observation suggests that the biosynthetic precursor is not the same for these two species or that moiety **1** is synthesized early in the development of the specimen. These suggestions can be further reinforced by considering the fact that *Vaccinium* (Ericaceae) and *Coreopsis* (Compositae) are not closely related.

EXPERIMENTAL

The phenolic fraction was isolated as follows: evaporation of the total methanolic extract of the liophylized material (1,2) *in vacuo*, followed by extraction with ether. The etheral fraction was backextracted with a 10% potassium carbonate solution. After removal of a large amount of salicyclic acid via basic extraction, a bright orange crystalline precipitate was isolated (0,017% of dry weight). The material turned deep red when treated with dilute alkali. The mass spectra of **2** were recorded on a Hitachi RM–50 at 70eV, 250° and on a MS901 for the exact mass measurement. Nmr spectra were recorded on a Varian XL–100 (^{1}H) and on a Varian FT–80A (^{13}C) in DMSO-d_6 (TMS at 0 ppm).

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