Synthese des Cantharidins in Larven. In 5 Larven des 2. Stadiums und je 4 Larven des 4. sowie des 5. Stadiums injizierten wir wässrige Natrium-^{14}C-1-acetat-Lösung. Nach 24 h wurden die Tiere getötet. Die Aufarbeitungsmethode für die Larven des 2. und 4. Stadiums unterschied sich von der bereits beschriebenen[3] darin, dass die Tiere nach Zugabe von 50 mg inaktiven Cantharidins nicht in Salzsäure, sondern in 2 *N* 80% alkoholischer Natronlauge 30 min unter Stickstoff gekocht wurden; damit wurde erreicht, dass die ebenfalls markierten Fette verseift und das Cantharidin anschliessend durch Extraktion aus sodaalkalischer Lösung von den Fettsäuren getrennt werden konnte.

Aus den in Tabelle II zusammengestellten Resultaten geht hervor, dass alle einzeln untersuchten Larven Cantharidin aufgebaut haben; die Einbaurate variiert allerdings in weiten Grenzen. Da die Wahrscheinlichkeit, dass bei einer Geschlechtsverteilung von 1:1 alle 7 der geprüften Larven Männchen waren (eine bald nach der Injektion gestorbene Larve des 4. Stadiums wird nicht berücksichtigt), nur 0,8% beträgt, ist anzunehmen, dass die Weibchen als Larven Cantharidin synthetisieren. Mit diesen Versuchen ist nicht nachgewiesen, dass die gesamte Cantharidinmenge adulter Weibchen larvaler Herkunft ist. Es wird zu klären sein, ob frischgeschlüpfte Adultweibchen noch Cantharidin bilden können, und ob bei der Kopulation auch Cantharidin vom Männchen auf das Weibchen übertragen wird. Letzteres ist nicht ganz ausgeschlossen, weil die Substanz in den Geschlechtsorganen beider Geschlechter angereichert ist[5].

Da Cantharidin sowohl in Adultweibchen als auch deren abgelegten Eiern und den daraus schlüpfenden *Triungulinus*-Larven vorkommen soll[5], wurde bisher angenommen, dass der in den *Triungulinus*-Larven nachgewiesene Stoff mütterlicher Herkunft sei. Dieser Schluss ist nicht unbedingt gerechtfertigt, da wir mit unseren Versuchen wahrscheinlich machen konnten, dass schon die Larven des 2. Stadiums Cantharidin selber synthetisieren. Die Abklärung der Verhältnisse bei *Triungulinus*-Larven ist uns noch nicht gelungen, da die kleinen, aber stark sklerotinisierten Tiere die Injektion nicht überlebten.

Über die biologische Bedeutung des Geschlechtsunterschiedes der Cantharidin-Synthese ist noch nichts bekannt[17].

Summary. Adult males of *Lytta vesicatoria* (*Coleopt., Meloidae*) synthesize cantharidin, but females do not, although the substance is present in the females too. We developed a method for rearing larvae of this parasitic beetle in the laboratory. It is shown that there exist 7 larval stages and 4 hypermetamorphic forms (*triungulinus*, second larva, *pseudochrysalis* and fourth larva). Larvae from the second to the fifth stage (second larva) synthesize cantharidin. Therefore, we conclude that the substance present in the adult female is at least in part synthesized in the larval period. Nothing is known about the biological significance of this biochemical sexual dimorphism.

DIETRICH MEYER, CH. SCHLATTER,
I. SCHLATTER-LANZ, H. SCHMID und P. BOVEY

Entomologisches Institut der Eidgenössischen Technischen Hochschule, 8006 Zürich und Organisch-Chemisches Institut der Universität, 8006 Zürich (Schweiz), 27. Juni 1968.

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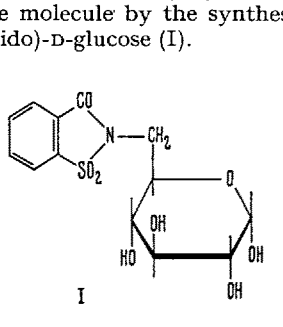
Synthesis and Taste Properties of an *N*-(Glucose)-saccharin Compound

It is generally concluded that substitution of the imide N-H in the saccharin molecule results in loss of sweetness[1,2]. The main reason for this might be the loss of water solubility, which would be true for most *N*-substituted derivatives. For instance, *N*-methyl saccharin would not be considered a valid study case, though its tastelessness was recently used[3] to suggest that the *N*-methyl prevents approach to the taste receptor site. Substituting a sugar, on the other hand, at the imide nitrogen should give a water-soluble derivative, which might then retain the sweetness of saccharin. Because it was thought that the glucose moiety, in addition to permitting water solubility, might enhance the sweetness or alter the bitter aftertaste of saccharin, these 2 sweetening agents have been combined in a single molecule by the synthesis of 6-deoxy-6-(*o*-sulfobenzimido)-D-glucose (I).



I

3,5-*O*-Benzylidene-1,2-*O*-isopropylidene-6-*O*-*p*-toluenesulfonyl-D-glucofuranose[4] was treated with sodium saccharin in dimethylformamide at 135° for 19 h. Upon concentration and dilution with water, a brown solid was obtained. After chromatography on a silica-gel column ($CHCl_3$ as eluting solvent) followed by crystallizations from isopropyl alcohol and ethanol, a 29% yield of 3,5-*O*-benzylidene-6-deoxy-1,2-*O*-isopropylidene-6-(*o*-sulfobenzimido)-D-glucose[5] was realized, as a broad-melting but analytically pure powder, $[\alpha]_D^{19}$ + 38.1° (*c* 0.52, chloroform). The benzylidene and isopropylidene blocking groups were removed by treatment with an acidic ion-exchange resin (Amberlite IR-120H) in 50% ethanol at 60–70°. A 71% yield of crystalline product was obtained, apparently a mixture of anomers of I, m.p. 180–187°, $[\alpha]_D^{25}$ + 36.3° (*c* 0.97, water) → + 32.7° (24 h). Two further recrystallizations from ethanol gave the α-anomer (I) as white needles[5], m.p. 204.5–206°, $[\alpha]_D^{21}$ + 42.1° (*c* 0.98, water) → + 32.7° (24 h). Water solubility had an upper limit of about 10%.

A 0.01 *M* aqueous solution of the compound was strongly bitter, with no appreciable sweetness, and an unpleasant aftertaste. The strong flavor indicated that there was good interaction of I with the receptor sites, at least with the

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bitter ones, despite the bulky *N*-substituent. The absence of sweetness may be related with some validity to the *N*-substitution, in terms of a recent hypothesis[6] that the system AH,B (where AH is a proton donor and B is a proton acceptor, of a certain proximity) is a prerequisite for sweetness. In saccharin, the AH group is the imide NH– missing from I.

This theory assumes that sweetness can be related to a particular structural feature (e.g. the AH,B system), rather than to overall properties of the molecule. If *N*-substitution made the saccharin moiety tasteless, one might expect a mild, sugar-like sweetness with I, since the AH,B group of the glucose moiety (C·1–OH, C·2–OH) remains intact. Apparently, any such effect of the sugar is overwhelmed by the much greater bitterness of I. The intensity suggests the bitterness is that inherent[7] in the saccharin structure, and only the sweetness of saccharin has been removed by *N*-substitution.

Résumé. La saccharine a été fixée par son azote imidique sur le C_6 du glucose, par l'action de la saccharine sodée sur un dérivé protégé du *p*-toluénesulfonyl-6-*O*-D-glucose, et élimination des groupes protecteurs pour former un composé hydrosoluble. La saveur amère peut être attribuée à l'élimination du proton imidique de la saccharine.

E. M. Acton, J. E. Christensen, H. Stone and L. Goodman

Life Sciences Research, Stanford Research Institute, Menlo Park (California 94025, USA), 17 June 1968.

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Studies in Medicinal Plants. Part III. Protoberberine Alkaloids from the Roots of *Cissampelos pareira* Linn.[1]

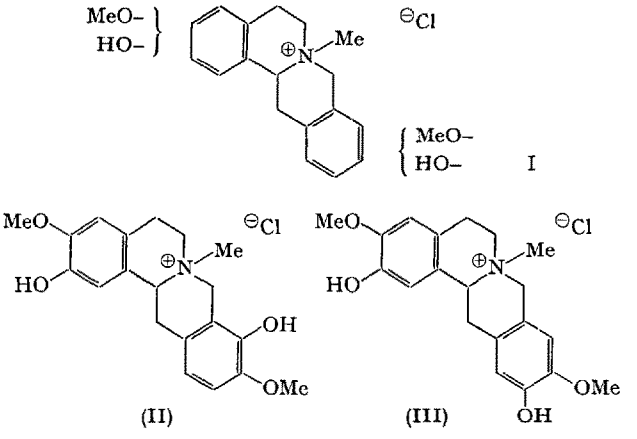
Our interest in the alkaloids from the roots of *Cissampelos pareira* Linn.[2–5] led us to the examination of its water-soluble quaternary bases previously isolated by two of us[5]. In the present communication only the essential data required to establish the constitution of Cissamine – the major quaternary alkaloid present in the roots of the plant – are reported.

Cissamine chloride has m.p. 215–220°, $[\alpha]_D^{26}$ – 129° (c = 1.00; MeOH). Its mass-spectrum revealed that the previous formula[5] was untenable; this has now been revised to $C_{20}H_{24}NO_4Cl$. Its UV-absorption at λ_{max}^{EtOH} 212, 235 and 285 nm (log ε 4.51, 4.10 and 3.94 respectively) corresponds to a tetrahydroberberine structure and the phenolic nature was shown by the spectral change in alkali to 218 nm (log ε 4.52) 253 nm (log ε 4.15) and 302 nm (log ε 4.04). The IR-spectrum supported the presence of phenolic hydroxyl group(s) (3509^{-1} cm).

The NMR-spectrum taken in D_2O on a Varian A-60 machine showed signals for 4 aromatic protons (3.02 to 3.15 τ), 1 $\overset{\oplus}{\text{N}}$-*Me* (6.75 τ) and 2 aromatic OMe groups (6.18 τ, 6.24 τ). There were 6 protons in the region 6.4–7.0 τ and 3 at τ 5.45.

A clue to the structure of cissamine chloride was provided by degradation studies. A double Hofmann degradation of the base chloride yielded a compound, m.p. 152–153° which contained 1 NMe_2 and 2 OMe groups. This, in conjunction with the foregoing data indicated that the N in this compound was in an environment as in tetrahydropalmatine and the partial structure of cissamine chloride could thus be written as (I). This was confirmed by the mass-spectrum of the quaternary base which showed the base peak at m/e 341 corresponding to the fragment M-HX (X = Cl)[6]. This narrowed the assignment of the structure of cissamine chloride to (II) and (III). The fixation of the positions of the 2 hydroxyls and 2 methoxyls in the 2 aromatic rings in these structures follows mainly biogenetic grounds.

A compound with the structure (II), named cyclanoline had recently been isolated from *Stephania tetrandra* by Tomita et al.[7]. A comparison of cissamine chloride with cyclanoline chloride (m.p., thin-layer chromatography, optical rotation and IR-spectrum) showed that the 2 compounds were, indeed, identical[8]. Structure II for cissamine chloride is thus confirmed.



Zusammenfassung. Cissaminechlorid aus den Wurzeln von *Cissampelos pareira* Linn. ist als Protoberberin-Alkaloid erkannt und mit Cyclanolinechlorid aus *Stephania terandra* identifiziert worden.

Falak Anwer, S. P. Popli, R. M. Srivastava and M. P. Khare

Central Drug Research Institute, and University, Lucknow (India), 11 June 1968.

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