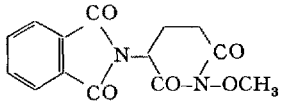
Relationship Between Teratogeny and Structure in the Thalidomide Field

It has been reported that the teratogeny of thalidomide is highly specific and that small structural changes destroy the teratogenic action of the related compounds, analogs as well as metabolites. Previous investigations of 14 related compounds revealed no relationship between sedative action and teratogeny, that both moieties of the molecule, glutamic acid and phthalic acid, are necessary for teratogeny[1,2], and that opening of one or both imide rings by hydrolysis leads to non-teratogenic metabolites[2-5]. Based on these findings, we synthesized the following 3 new analogs, and were surprised to find 1 of them (WU-385) to be significantly active.

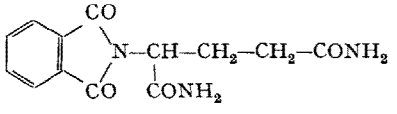
(1) WU-362 = *N*-methoxythalidomide. Earlier investigations had shown that *N*-methylthalidomide (WU-313, 2-phthalimido-*N*-methyl-DL-glutarimide) is teratogenic[1], possibly due to a demethylation in vivo. If the hydrogen of glutarimide is replaced by methoxy, the possible demethylation would lead to *N*-hydroxythalidomide (WU-347), a compound without teratogeny in rabbits (WUEST and FRATTA, unpublished results).

For the synthesis of *N*-methoxythalidomide, phthaloyl-DL-glutamic anhydride was reacted with methoxamine and the resulting methoxamide was treated with acetic anhydride to close the ring: WU-362-*N*-methoxythalidomide = 2-phthalimido-DL-*N*-methoxyglutarimide, white crystals, m.p. 245°.

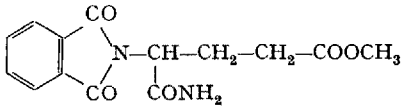


(2) WU-380 = 2-phthalimido-DL-glutaric diamide, an open chain without carboxyl groups. All known metabolites of thalidomide are formed by hydrolysis and therefore, with the exception of α-aminoglutarimide, contain carboxyl groups[6,7]. KEBERLE[4] believes that the lack of teratogeny is due to the presence of ionizable carboxyl groups and the consequent inability of these metabolites to penetrate the placenta. However, if we open the glutarimide ring with ammonia or methanol, thalidomide analogs with substituted carboxyl groups are obtained.

Phthaloyl glutamic acid was converted with phosphorous pentachloride to its dichloride (m.p. 76–77°) which on treatment with ammonia in ether yielded WU-380 = 2-phthalimido-DL-glutaric diamide, white crystals, m.p. 217–218°.



(3) WU-385 = methylester of *N*-α-phthaloyl-DL-isoglutamine = methyl-4-phthalimido-DL-glutaramate.



γ-Methylphthaloyl-DL-glutamate (m.p. 119°) was chlorinated with phosphorous pentachloride and the acyl chloride treated with ammonia; the resulting ester-amide melted at 141° after recrystallization and is isomeric to the ester-amide of SHEEHAN and BOLHOFER with the m.p. of 143–144°[8]. The mixture m.p. showed a depression of about 20°.

It is the purpose of this communication to report the results of testing these 3 compounds, WU-362, WU-380 and WU-385 on pregnant New Zealand White rabbits.

Forty litters were obtained from 20 strain III females. Each doe produced both a treated and a control litter and was bred to the same male for both litters. The compounds were administered orally in gelatin capsules at the rate of 150 mg/kg body weight on days 6–11 of gestation. We considered the day of mating as day zero. Capsules were given by a balling gun so that swallowing was immediate with negligible trauma[9]. Matings for control and treated litters were made to avoid bias due to season or parity. Pregnancy was determined by palpation[10] 10–14 days after coitus. If parturition failed to occur by day 32, it was induced by oxytocin. Both the hormonal induction of parturition and the examination of new-borns for malformations were in the manner of SAWIN et al.[9]. Control and treated litters did not differ in litter size at the 0.05 level of significance (Table).

Neither WU-362 nor WU-380 have any significant effect on malformation of the rabbit fetus. However, a significant increase in malformation ($P < 0.01$) is seen in the Table when WU-385, an ester of phthaloyl-DL-isoglutamine, was administered. The abnormalities observed were primarily of soft tissues (megacolon, absent kidneys, aortic stenosis and diverticulum of colon), but some fused vertebrae were noted. We observed these soft tissue abnormalities in thalidomide-treated rabbits[9] with a similar frequency and feel certain therefore that they are truly part of the thalidomide syndrome. They do not reflect, however, the usual or major skeletal effects. Therefore, the teratogeny of WU-385 is certainly less extensive than that of thalidomide.

Since the ring closure of WU-385 to thalidomide can be enforced by strong alkali in absence of water (equimolar

The effects of oral administration of thalidomide analogs on malformation of rabbit fetuses

Treatment	Litters	Classifiable fetuses (No.) Total	Weighted Abnormal[a] (%)	Unclassified Resorbed Fetuses (No.)
WU-362	7	40	1 (2.5) N.S. $P > 0.05$	2 N.S. $P > 0.05$
Control	7	44	1 (2.3)	3
WU-380	8	40	2 (5.0) N.S. $P > 0.05$	2 N.S. $P > 0.05$
Control	8	44	1 (2.3)	0
WU-385	5	24	10 (41.7) $P < 0.01$	2 N.S. $P > 0.05$
Control	5	38	1 (2.6)	3

Pregnant rabbits were given compounds on days 6–11 of gestation, 150 mg/kg day. [a] Those animals having minor malformation of skull, sternum, or tail were classified as normal since this is considered to be normal strain variation[9].

[1] H. M. WUEST, E. B. SIGG and I. FRATTA, Life Sci. *3*, 721 (1964).
[2] H. M. WUEST, I. FRATTA and E. B. SIGG, Life Sci. *5*, 393 (1966).
[3] S. FABRO, H. SCHUHMACHER, R. L. SMITH, R. B. L. STAGG and R. T. WILLIAMS, Biochem. J. *90*, 5 (1964).
[4] H. KEBERLE, P. LOUSTALOT, R. K. MALLER, J. W. FAIGLE and K. SCHMID, Ann. N.Y. Acad. Sci. *123*, 252 (1965).
[5] H. FRITZ, J. Reprod. Fert. *11*, 157 (1966).
[6] J. W. FAIGLE, H. KEBERLE, W. RIESS and K. SCHMID, Experientia *18*, 389 (1962).
[7] R. T. WILLIAMS and D. V. PARKE, A. Rev. Pharmac. *4*, 91 (1964).
[8] J. C. SHEEHAN and W. A. BOLHOFER, J. Am. chem. Soc. *72*, 2472 (1950).
[9] P. B. SAWIN, D. D. CRARY, R. R. FOX and H. M. WUEST, Experientia *21*, 672 (1965).
[10] A. E. SUITOR, USDA Leaflet 245 (1946).

amounts of sodium methylate in dimethylformamide, yield 81%), we cannot exclude its transformation into thalidomide in vivo (e.g. in the alkaline milieu of the intestine). If this ring closure of ingested WU-385 takes place, it would be expected to occur only to a small extent because skeletal malformations, frequently observed with thalidomide, were found only to a very slight degree. It will be interesting to see if the internal malformations observed earlier by us with high doses of thalidomide[9] will also occur with doses small enough not to produce skeletal effects[11,12].

Zusammenfassung. Thalidomid verursacht an trächtigen Neuseeland-Kaninchen nach oraler Verabreichung starke Missbildungen des Wurfes. Geringe strukturelle Abweichungen können die Teratogenie zerstören: Zwei solche Verbindungen, *N*-Methoxy-thalidomid und 2-Phthalimido-glutaramid, sind nicht teratogen, während ein Estermid, ein Thalidomidanalog mit offener Kette, hauptsächlich Missbildungen der inneren Organe erzeugt.

H. M. WUEST, R. R. FOX and D. D. CRARY

Sloan-Kettering Institute for Cancer Research, New York City (N.Y. 10021) and The Jackson Laboratory, Bar Harbor (Maine, USA), 13 June 1968.

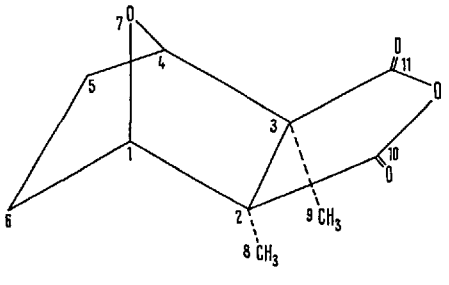
[11] The principles of laboratory animal care as promulgated by the Council of the American Physiological Society are observed in this Laboratory.

[12] This research was supported in part by Public Health Service Research Grants, No. HD-01496 from the National Institute of Child Health and Human Development and No. FR-00251 from the Division of Research Facilities and Resources to The Jackson Laboratory, Bar Harbor, Maine; and Public Health Service Grant No. CA-08748 to The Sloan-Kettering Institute for Cancer Research.

Zur Biosynthese des Cantharidins. I

Im Rahmen unserer Untersuchungen über die Biochemie von Insekteninhaltsstoffen haben wir die Abklärung der Biosynthese des Cantharidins in Angriff genommen. Cantharidin ist ein Inhaltsstoff des Käfers *Lytta vesicatoria* (L.) (Coleopt. Meloidae), der sogenannten spanischen Fliege, welche früher in Human- und Veterinärmedizin als Blasenzugsmittel häufig gebraucht wurde und heute noch gelegentlich bei fälschlicher Verwendung als Aphrodisiacum zu Vergiftungen Anlass gibt. Der Wirkstoff wurde im Jahre 1810 von ROBIQUET[1] in kristallisiertem Zustande erhalten. Die Strukturaufklärung hat man GADAMER[2] zu verdanken. Totalsynthesen publizierten die Arbeitsgruppen von ZIEGLER und SCHENCK[3] sowie von STORK[4].

Cantharidin



Die auf der Hand liegende Hypothese der Cantharidin-Biosynthese besteht in einer Schwanz-Schwanz-Verknüpfung von 2 Isopreneinheiten. Gegen diesen Aufbauweg ist allerdings einzuwenden, dass eine solche Verknüpfung zwar bei der Bildung von Squalen aus 2 Farnesylpyrophosphat-Molekeln, nie jedoch auf der Stufe von 2 einzelnen Isopreneinheiten gefunden wurde[5], obwohl die strukturelle Variabilität der Isoprenabkömmlinge äusserst gross ist.

L. vesicatoria kommt in der Schweiz sehr selten vor. In den Mittelmeerländern tritt das Tier im Frühsommer jeweils während ca. 2 Wochen an bestimmten, leider nur unsicher vorauszusagenden Stellen oft massenhaft auf. Es gelang indessen, im Juli 1966 im Simplongebiet 14 Käfer zu sammeln[6].

In Äthernarkose injizierte man 6 Tieren (4 ♀ und 2 ♂) insgesamt 1 mc einer Natrium-^{14}C-1-acetat-Lösung (1,5 mg in 0,1 ml Wasser [$c = 0{,}18\,M$]) und 8 Tieren (5 ♀ und 3 ♂) 0,58 mc einer Natrium-SR-^{14}C-2-mevalonat-Lösung (25 mg in 0,12 ml Wasser [$c = 1{,}1\,M$]). Nach 10–24 h wurden die Tiere nach Zugabe von 10 mg inaktivem Cantharidin pro Tier mit Sand zerrieben, in 1 *N* wässeriger Salzsäure 1 h gekocht und dann das Ganze kontinuierlich mit Benzol extrahiert. Aus dem Extrakt erhielt man nach Chromatographie, mehreren Umkristallisationen, Hochvakuumsublimationen und Behandlung mit 2 *N* Natronlauge als weiterer Reinigungsoperation schliesslich Cantharidin von konstanter Aktivität. Es zeigte sich, dass weibliche Tiere nicht imstande waren, radioaktives Cantharidin in messbarer Menge zu synthetisieren, obwohl Versuche an anderen Lyttaweibchen ergaben, dass diese praktisch ebensoviel Cantharidin enthielten wie die Männchen, nämlich 0,5–1%. Die Inkorporierungsrate bei Männchen betrug in beiden Versuchen je ca. 0,2%.

Im radioaktiven Cantharidin wurden die C-Atome 10 + 11 durch Schmidt-Abbau[7] erhalten. Durch Kuhn-Roth-Oxydation (Gef. 1,4 CH_3[C]) konnten die C-Atome 2 + 8 + 3 + 9 in Form von Essigsäure isoliert werden, welche als *p*-Bromphenacylester ausgezählt wurde. Ein Teil der Essigsäure wurde durch Pyrolyse des Lithium-Salzes zu Lithiumcarbonat (C-Atome 2 + 3) und Aceton

[1] M. ROBIQUET, Annls Chim. *76*, 302 (1810).

[2] J. GADAMER, Arch. Pharm., Berl. *260*, 199 (1922); sowie 10 frühere Mitteilungen in Arch. Pharm., Berl. *252–260*.

[3] K. ZIEGLER, G. W. SCHENCK, E. W. KROCKOW, A. SIEBERT, A. WENZ und H. WEBER, Justus Liebigs Annln Chem. *551*, 1 (1942). – G. W. SCHENCK und R. WIRTZ, Naturwissenschaften *40*, 581 (1953).

[4] G. STORK, E. E. VAN TAMELEN, L. J. FRIEDMAN und A. W. BURGSTAHLER, J. Am. chem. Soc. *75*, 384 (1953).

[5] Vgl. z.B. J. H. RICHARDS und J. B. HENDRICKSON, *The Biosynthesis of Steroids, Terpenes and Acetogenins* (W. A. Benjamin, New York 1964).

[6] Wir danken Herrn Dr. V. ALLENSPACH, Wädenswil, sehr für die Angabe dieses Fundortes.

[7] Der Schmidt-Abbau musste mit konz. Schwefelsäure und Natriumazid bei 130 °C im Hochvakuum durchgeführt werden; mit Polyphosphorsäure trat bis 150 °C keine Reaktion ein.