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EXTRAS

2

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Fire Ant Carbohydrate Phagostimulants : Laboratory and Practical Studies

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The fire ant, *Solenopsis invicta* was accidently imported into the United States in the 1930's from South America. Since then it has infested nine southern states and Puerto Rico (approximately 150 million hectares) (1). It has the potential to spread into Arizona, California, Oregon and Washington : however, the deserts of Texas and stringent quarantine measures currently hold it in chek (2).

Mature fire ant colonies contain up to 250 000 workers and 120 colonies may infest each hectare. The ant is considered a pest for medical and agricultural reasons (3). The highly aggressive workers have a potent sting and the injected venom has a wide variety of physiological effects, the most severe of which is hypersensitivity (4). The stings are painful and may curtail people's outdoor activities.

Fire ant workers attack a wide variety of crops including soybeans, potatoes, corn, citrus, and okra. They feed on germinating seeds in corn and soybeans fields, thus decreasing crop yield (3). This kind of demage previously went unnoticed because all activity was underground. However, in okra the demage done by ants is visibly evident. The worker ants macerate the sepal area of the flower and presumably feed on the plant fluids. Severely attacked flowers may produce deformed fruit or no fruit.

We report here the results of carbohydrate phagostimulant tests, as well as okra and soybean carbohydrate analyses that help explain the observed fire ant attacks on these plants.

MATERIALS AND METHODS

Source of Colonies. Laboratory colonies of *S. invicta* were reared from newly mated queens collected near Gainsville, Florida using standardized procedures (5).

Carbohydrate Phagostimulant Bioassay. The bioassay was similar to one already decribed (6), except Whatman Phase Separator (hydrophobic) filter paper squares (2×2 cm) were used to present the aqueous test carbohydrates to the ant.

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All carbohydrates tested (from Sigma Chemical Company, St. Louis, Missoury or Calbiochem, La Jolla, California) were prepared as one percent (W/V) aqueous solutions. Samples (100 uL) were applied to the center of the phase separator filter paper squares and then randomly placed around ten symmetrical locations on the tray floor of the laboratory colony.

The bioassay was evaluated by counting the number of ants feeding at the droplet every 10 minutes for a total of 60 minutes. The results for the six time periods were added and the total used to calculate the ranking. A water control and sucrose standard was included in each test. The test samples were ranked by setting the water response at zero and the sucroseresponse at 100. The ranking was calculated as follows:

Response (sample) — Response (H_2O)×100

Response (sucrose) — Response (H_2O)

The mean and standard error for five replicates were calculated for each sample.

Bioassay for Okra Sepal Fractionation. The bioassay was similar to one already described (5), except the number of ants within the treated area was recorded every half hour for a total of three hours. The results for each fraction, control, and standard were totaled and the percent response calculated for each. The mean response and standard deviation were calculated for the five replicates. The standard was the precursor to the tested fractions and the solvent was the control.

Okra Sepal Fractionation. The sepal area of okra flowers was sliced several places with a razor blade while on the plant and the whole flower collected 10 minutes later. Samples were frozen until used. Just prior to use the flower petals were removed and the sepal and part of the stem homogenized in a Waring blender with 60% aqueous ethanol. The extraction was repeated five time. The residue was extracted three times with chloroform. The combined aqueous alcohol extract was vacuum evaporated until an aqueous solution remained. This solution was extracted three times with chloroform to remove lipids. The aqueous (A) and combined chloroform (B) extracts were bioassay. The active aqueous extract (A) was further fractionated by passing an aliquot through a column of Dowex 50X-8 strongly acidic resin. Neutral and acidic components (D) were eluted with water, then amino acids and other compounds were eluted with 4N NH_4OH. An additional fraction (E) was abtained by eluting the column with methanol. All fractions were reduced to the volume of the active precursor (in this case aqueous fraction A). All fraction were tested in the bioassay. An aliquot of the active neutral-acidic fraction (D) was passed through a bosic Sephadex-25 ion exhange resin, which on elution with water retained acidic compounds (F) and allowed neutral materials to pass through. One neutral fraction was collected (G), and found to be strongly positive for reducing carbohydrates.

GC Analysis of Carbohydrates. An aliquot of each sample to be analyzed was evaporated and an equal volume of Tri-Sil Z (trimethylsilylimidazole in dry pyridine, 1.5 mEq, Pierce Chemical Company, Rockford, Illinois) was added. Each sample was heated at 70 C for 45 minutes with a vortex treatment at 30 minutes.

Derivatized carbohydrates were analyzed by GC on a Varian 3 700 equipped with a flame ionization detector (Varian Associates, Palo

Alto, California) and a 1,8 m×2-mm i.d. glass column packed with 3% OV-17 on 120/140 mesh Gas-Chrom (Applied Science Laboratories, State College Pennsylvania). The GC oven temperature was programmed from 100 to 250 C at 10 C/min. Qualitative and quantitative data were obtained with a Varian Vista 401 data processor. Sucrose, glucose, and fructose standards for GC analysis were purchased from Calbiochem and Sigma Chemical Company. Quantitative data were obtained using a trehalose internal standard, and peak identification was made by direct GC comparison with silyated carbohydrate standards.

RESULTS AND DISCUSSION

Carbohydrate Phagostimulant Tests. Of nine naturally occurring monosaccharides only glucose, fructose and L-fucose had significant phagostimulant activity (fig. 1). All others had activity either indistinguishable or below that of the water control. The glucose result corresponded to the sucrose standard. The opposite enantiomers (unnatural) of the phagostimulants, glucose and fucose were inactive, as were those of mannose and arabinose (fig. 2). These data emphasize the ant's discriminatory capabilites and the lock and key concept of phagostimulant substrate/receptor site. Phagostimulant tests conductel with seven modified glucose compounds (fig. 3) further illustrated this concept. In all cases modifying the glucose molecule resulted in decreased activity. Only 2-deoxy-2-fluoro-glucose, 5-thio-glucose and glucose-1, 6, diphosphate had mean activity scores above that of the water control.

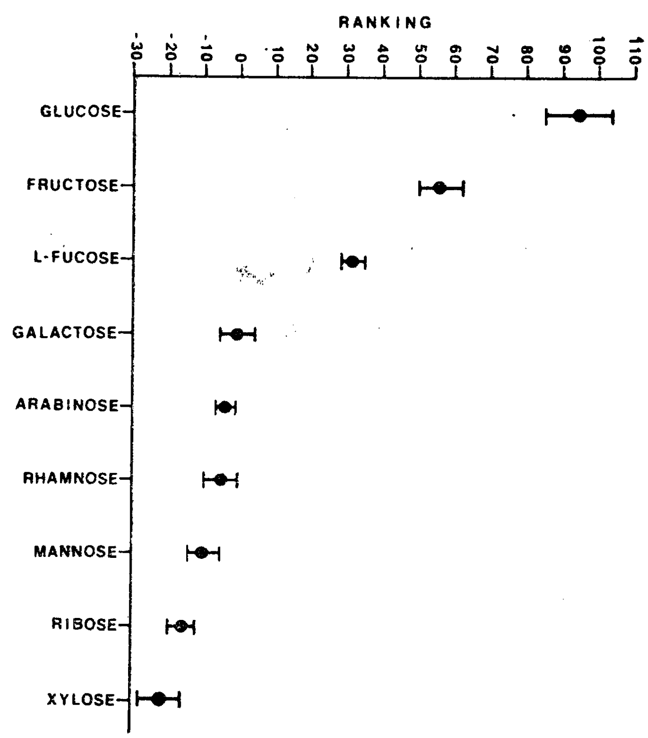

Figure 1 — Phagostimulant bioassay results for monosaccharides (Ranking based on sucrose = 100; and water=0).

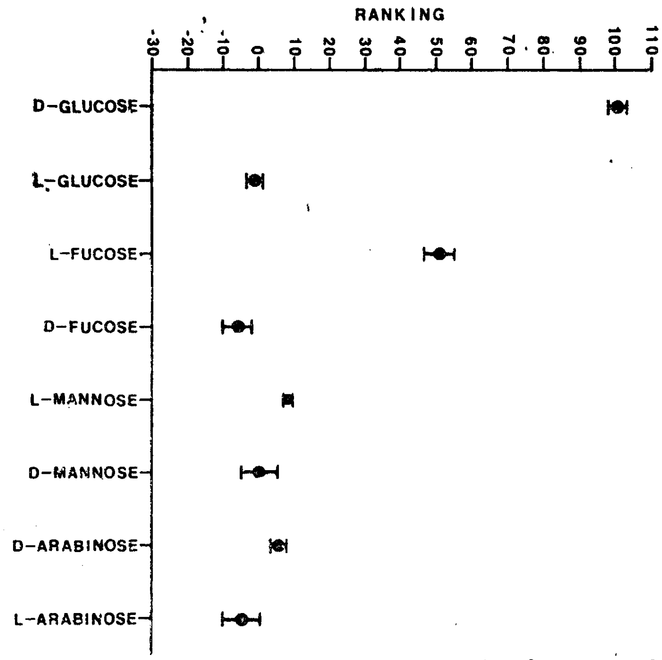

Figure 2 — Phagostimulant bioassay results for naturally occurring monosaccharides and their diasteriomers (Ranking based on sucrose = 100; and water = 0).

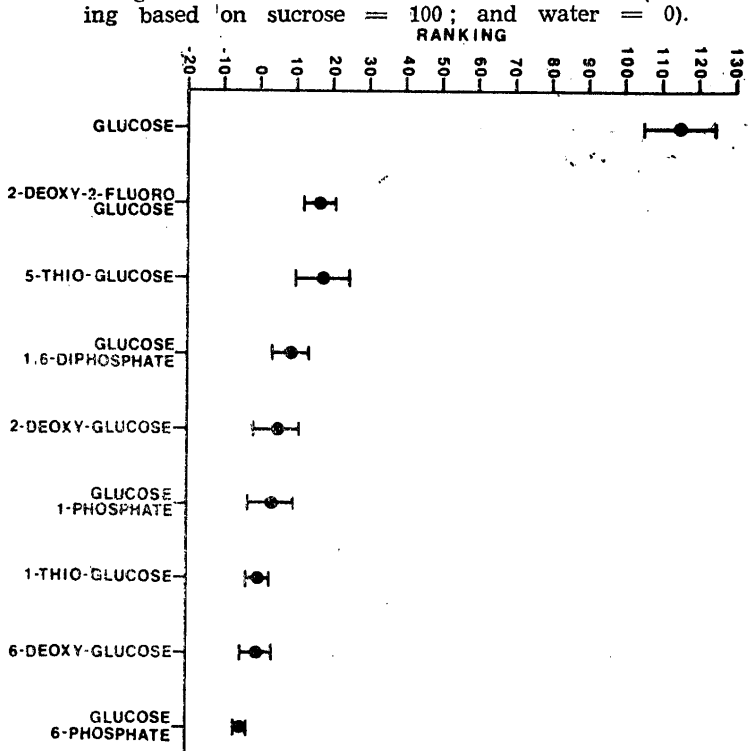

Figure 3 — Phagostimulant bioassay results for glucose and its derivatives (Ranking based on sucrose = 100; and water = 0).

Three of the seven disaccharides tested (including sucrose) had excellent phagostimulant activity (fig. 4). Maltose had activity equal to that of the sucrose standard, while turanose was only slightly below the standard. Only one trisaccharide, raffinose, was tested, and it had a good phagostimulant activity, although less than the active disaccharides.

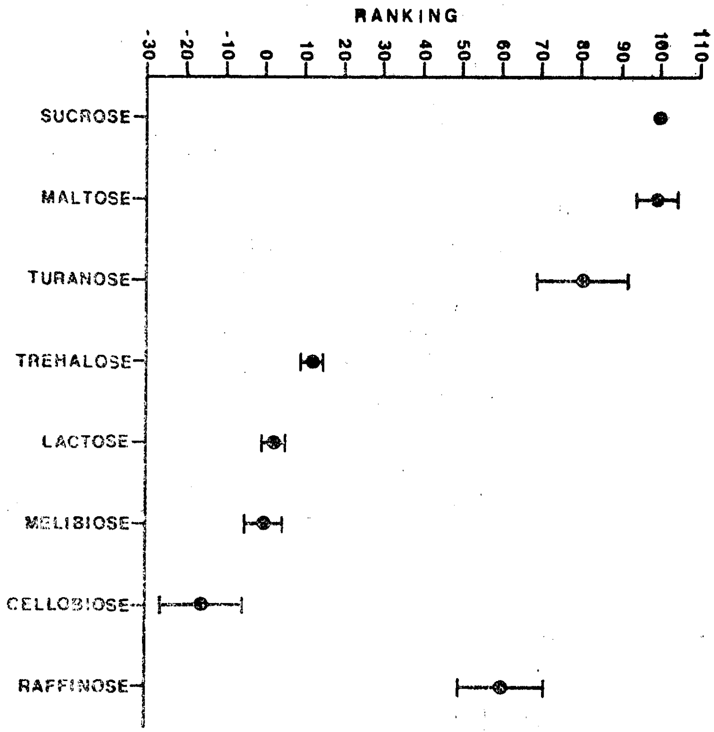

Figure 4 — Phagostimulant bioassay results for disaccharides and one trisaccharide (Ranking based on sucrose = 100 ; and water = 0).

Nine sugar alcohols were tested (fig. 5). Only myoinositol had greater activity than the water control. It ranked far below the phagostimulant activity of the sucrose standard (13 vs. 100).

These tests reveral that *S. invicta* can distinguish, by taste, a wide variety of carbohydrates and sugar alcohols. The carbohydrates which act as strong phagostimulants may play important roles in the ant's choice of food, especially plant sources.

Okra Phagostimulant. Fire ants macerate only the sepal area of okra flowers, but no other plant parts (3). The adult ants ingest only liquids and have a sophisticated filtration mechanism (7). Consequently they probably feed on fluids exuded from the damaged sepal. Since the ants have an equal opportunity to attack other plant parts, phagostimulants may be associated with the okra sepal. Extraction of okra sepals with 60% aqueous ethanol (A) and chloroform (B) gave polar and lipid fractions, respectively (fig. 6). Lipid fraction (B) was significantly better than the solvent control but much less active than the aqueous fraction Consequently, fraction (A) was further searated into neutral/acidic components (D) and basic/amino acids components (C). Bioassay results clearly showed fraction (D) equivalent to the precursor fraction (A). Fraction (C) was inactive, indicating that amino acids were not phagostimulantes. Fractionation of (D) through basic sephadex 25 resin retained acidic compounds and gave neutral fraction (G). This fraction (G) had activity equivalent to parent fraction (D) and tested positive for reducing carbohydrates. Derivatization of an aliquot and analysis by GC revealed the presence of significant fructose, glucose and sucrose levels. These results coupled with our previous carbohidrate investigations led to a quantitative study of the sugar content of various okra plant parts. The okra sepal contains almost five times more carbohydrate than okra stalks, leaves or fresh seeds. All three sugars identified (fructose, glucose and sucrose) are strong fire ant phagostimulants. Based on the sepal water content the amount of carbohydrate is greater than 3 percent : three times greater than that used in laboratory phagostimulant studies. The other plant parts contain less than 1.0 percent carbohydrate, still adequate to stimulate feeding. Even more enigmatic is the fruit's high carbohydrate content sans seeds. The okra sepal contains almost five times more carbohydrate than okra stalks, leaves or fresh seeds. All three sugars identified (fructose, glucose and sucrose) are

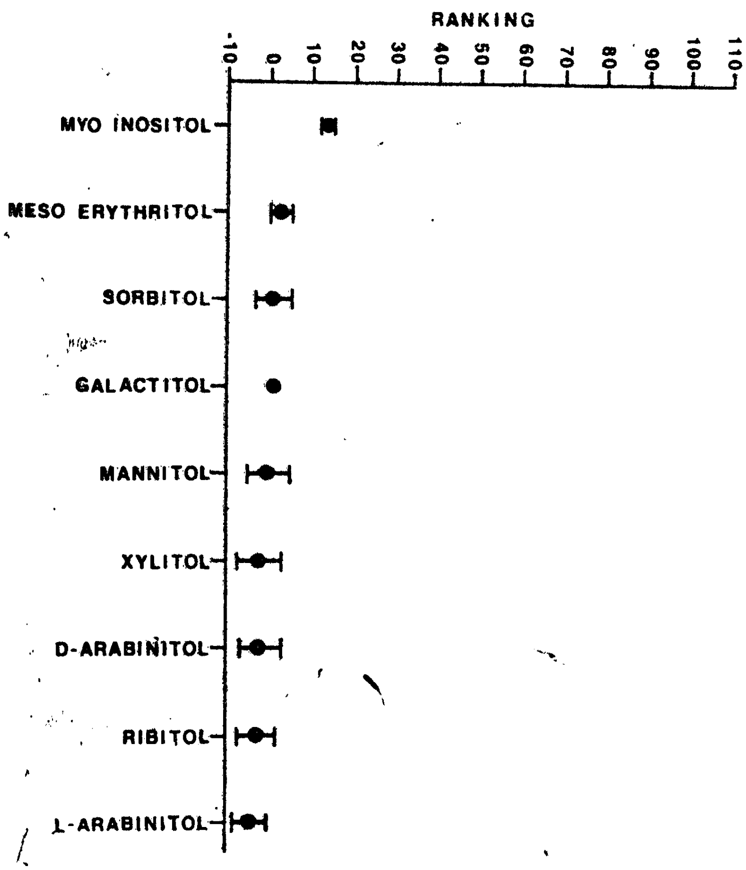

Figure 5 — Phagostimulant bioassay results for several sugar alcohols (Ranking based on sucrose = 100 ; and water = 0).

strong fire ant phagostimulants. Based on the sepal water content the amount of carbohydrate is greater than 3 percent ; three times greater than that used in laboratory phagostimulant, studies. The other plant parts contain less than 1.0 percent carbohydrate, still adequate to stimulate feeding. Even more enigmatic is the fruit's high carbohydrate content sans seeds. Although the results appear contradictory, they reflect another important factor, accessibility. The ants cannot get at the carbohydrates in the tough stalk, leaves or fruit, but the soft sepal is easily macerated. The sepal has high amounts of carbohydrates due tot nectaries. Many plant species have extrafloral nectaries, which attract ants and/or other insect (8). The ants benefit, from the easily obtained carbohydrates, and the plant benefits by the negative influence ants have on potential herbivores (9). In spite of the damage done to the sepal, the fruit and seeds do develop ; however, the deformed fruit no longer has commercial value.

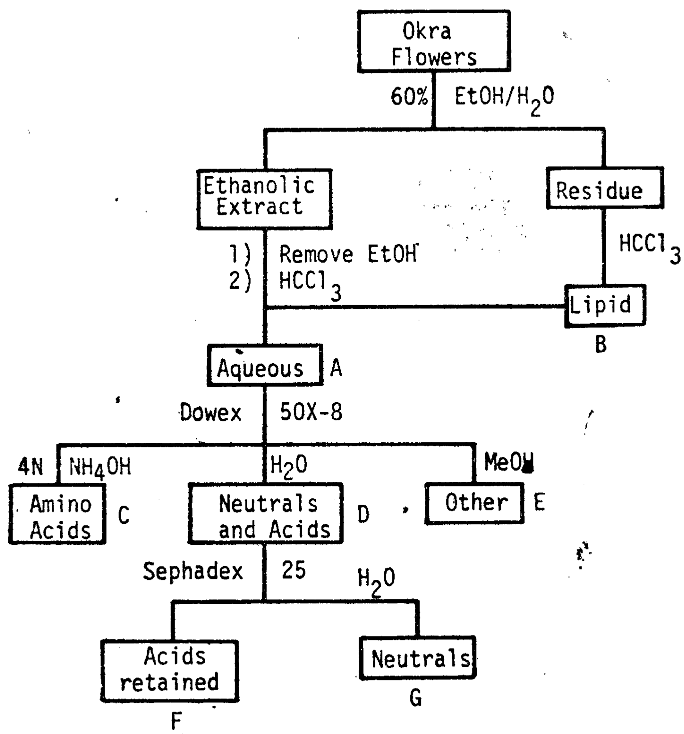

Figure 6 — Flow diagram for the isolation of a fire ant phagostimulant from okra.

Soybeans. Fire ants significatly reduce the yield of soybeans, a major crop in the southern United States (10). The ants damaged germinating seeds ; once thes plants developed a few leaves the ants had no apparent effect on subsequent growth, although radio tracer studies revealed that the ants do feed on the growing plant (10). The net result was a significant decrease in the number of plants per row. Based on our previous work with carbohydrate phagostimulants and okra, we investigated the sugar content of soybeans sprouts and mature plant parts.

The results show that sprouts contain more phagostimulant carbohydrates than roots, stems, or leaves from mature plants. As discovered with okra, all plant parts do have significant amounts of carbohydrates ; however, roots, stems and leaves are much more difficult to penetrante than soft tender sprouts. In addition, field preparation for soybean planting eliminates much of the previously existing food material, including insects and other plants. Consequently, the opportunistic fire ant takes advantage of soft tissued sprouts to maintain itself until insect fauna population levels increase.

We have demonstrated that several common plant carbohydrates stimulate fire ants to feed, and that these materials may play a role in host selection. Okra flowers have extrafloral nectaries around the sepal area, which induce feeding and recruitment by fire ant workers. The feeding action damages the sepal and results in deformed fruit which, although not detrimental to the plant ruins their commercial appearance. The ants attack soybean sprouts because of their soft tissue, high carbohydrate content, and scarcity of alternative food materials. Both examples illustrate the opportunistic nature of the fire ant. Undoubtedly we will discover further examples of negative interactions between man and fire ants.

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REZUMAT

Robert K. Vander Meer, Emanuel Merdinger — HIDRAȚII DE CARBON, FAGOSTIMULENȚI AI FURNICII DE FOC ; STUDII DE LABORATOR ȘI PRACTICE

Furnica de foc era considerată în trecut o mare mîncătoare de petrol. Astăzi s-a stabilit că ea necesită o hrană diversificată, incluzînd hidrați de carbon. Printr-un screening cuprinzător asupra a numeroși hidrați de carbon s-a constatat că doar un număr limitat au un efect fagostimulant (adică determină fur-

nicile să se hrănească). Între aceste substanţe se numără şi monozaharide (glucoza, fructoza, l-fucoza) şi dizaharide (sucroza, maltoza şi furanoza). Prin modificarea glucozei foarte active s-a obţinut dispariţia efectelor fagostimulante: s-a conchis că activitatea respectivă este foarte sensibilă la structura moleculei. Astfel autorii au putut înţelege mai bine de ce furnica de foc atacă doar anumite tipuri de plante. Anumite părţi ale florilor de bame sînt atacate de ele, ceea ce duce la deformarea fructelor. Analiza chimică a hidraţilor de carbon din diferite părţi ale plantei a demonstrat că furnica de foc atacă acele părţi ale plantei care conţin o cantitate de hidraţi de carbon de cinci ori mai mare decît alte părţi.

РЕЗЮМЕ

Роберт К. Вандер Меер, Емануел Мердингер — ГИДРАТЫ УГЛЕРОДА ФАГОСТИМУЛЕНТЫ ОГНЕВОГО МУРАВЕЯ, ЛАБОРАТОРНЫЕ И ПРАКТИЧЕСКИЕ ИССЛЕДОВАНИЯ

В прошлом считалось что огневые муравая кормились нефтью и сегодня узнавается что они требуют разнообразное кормление, включая углеролистые гидраты. Осуществление скреенинга на большом числе улеродистых гидратов привело к утверждению что только часть проверяемых имеют фагостимулирующее действие для огневых муравей (провоцирую кормится). Они содержит моносахариды глюккозы, фруктозу и Л-фруктозу, и дисахариды сукрозы, малтозы и туранозы. Всякое изменение в структуре силно активнои глюккозы привело к отсутствии фагостимулирования, так что деятельность очень чуствительное к форме молекулы. Это разрешило нам понимать почему огневые муравя нападают на некоторые уражая. Чашелистники цветов бамии атакированы огневыми муравями, что приводит к деформацию фрукта. Химический анализ гидратов углерода в разных частеи растения показывал что они содержили приблизительно пять раз количество гидратов углерода фагостимулирующие.

REFERATE

EFECTELE VASODILATATOARE ALE UNUI NOU DERIVAT DE 1,4 DIHIDROPIRIDINĂ, COMPARATIV CU NIFEDIPINA, **D. Pruneau, F. Roy, N. L. Brown**, Therapie, 1989, 44, 3, 203—208

Sinteza substanţelor derivate de la 1-4 dihidropiridină a condus la obţinerea de medicamente deosebit de valoroase pentru terapia bolilor aparatului cardiovascular.

Din grupa blocantelor transportului ionilor de calciu nifedipina este mult folosită în afecţiuni cardiace, ca şi nisoldipina, iar nicardipina sau nimodipina au o mare specificitate ca vasodilatatoare cerebrale. Pentru alte substanţe cum sînt: amlodipina, benidipina şi lacidipina s-a stabilit un efect de întîrziere a intrării ionilor de calciu de lungă durată atît în testările făcute *in vivo* cît şi *in vitro*.

Autorii testează efectele farmacodinamice ale unui nou compus denumit convenţional LF 2 0254, compus care blochează intrarea calciului în muşchiul neted vascular, ca urmare a unui influx redus în canalele lente ale ionilor respectivi, iar asupra miocardului efectul este mai slab decît cel produs de nifedipin sau verapamil.

În concluzie, derivatul nou de 1, 4 dihidropiridină prin efectul vasodilatator intens şi de lungă durată pare mai avantajos decît alte substanţe înrudite cum sînt: lacidipina, benidipina, oxodipina sau amlodipina.

Elena Cristea

EFECTUL PROTECTOR AL UNEI FRACŢIUNI FLAVONICE PURIFICATE ASUPRA TULBURĂRILOR DATE DE APARIŢIA RADICALILOR LIBERI DE OXIGEN, **M. Lonchampt, B. Guardiola, N. Sicot, N. Bertrand, L. Pedrix, J. Duhault**, Arzneimittel forschung, 1989, 39, II, 8, 882—885

Apariţia de radicali liberi de oxigen activ este implicată în multe stări patologice cum sînt inflamaţiile, ateroscleroză, carcinogeneză, ischemie cerebrală sau cardiacă, ulcerații ale mucoaselor gastrică sau duodenală, opacifieri ale cristalinului, îmbătrînirea diferitelor organe.

Plecînd de la aceste date, numeroși autori se preocupă de găsirea de substanțe capabile să intervină în formarea și neutralizarea acestor produse endogene atît de dăunătoare organismului uman. Știindu-se că o serie de derivați flavonici au proprietăți antiinflamatorii și pentru ei s-a mai pus în evidență și o influență asupra radicalilor liberi, s-a realizat o asociere de diosmină 90% și hesperidină 10%, respectiv de două fracțiuni flavonoidice aduse la un grad avansat de purificare.

Preparatul aflat sub formă de comprimate (Daflon Franța) cercetat *in vitro* și *in vivo* s-a dovedit activ asupra radicalilor liberi de oxigen apăruți în timpul fagocitozei sau în cursul dezvoltării diabetului experimental prin injectarea de aloxan.

Efectul protector al asocierii de flavone explică diversele proprietăți farmacologice ale preparatului respectiv, cum sînt scăderea permeabilității capilarelor indusă prin iradiere, injectare de antigeni nespecifici sau aplicare de cloroform, dar și acțiunea antiinflamatoare și antiedematoasă.

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