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NYCTINASTENES - AN APPROACH TO NEW PHYTOHORMONES

Hermann Schildknecht and Kurt Schumacher

Organisch-Chemisches Institut der Universität Heidelberg,
Im Neuenheimer Feld 27o, 69oo Heidelberg, Germany

Abstract - A plant responds to an irritation by releasing irritants which may assist the organism endogenously in an intrinsic defence reaction. Only very small energy changes perceived by sensitive plants are sufficient to induce a series of physiological processes ultimately manifested as leaf movement. The chemonastic active compounds of sleeping and sensitive plants - the Leaf MovementFactors Factors LMF - are factors only in a stimulation chain that definitely also comprises amino acids and possibly even an inhibitor,D-pinitol. The active fraction from Acacia karroo characterized by the Fitting-Hesse-Schildknecht-test with the sensitive plant Mimosa pudica L. contains, besides the three Leaf Movement Factors K-LMF 1, 2 and 3, the Periodic Leaf Movement Factors K-PLMF 1 and 2. The assigned structure of these new phytohormones with the name "Nyctinastenes" was confirmed by syntheses. The K-PLMF 1 is the 4-O-[3,5-Dihydroxybenzoic acid]ß-D-glucosid-6'-sulfate.

DARWINIAN MOVEMENTS OF PLANTS

A hundred years ago, in 188o, "The Power of Movement in Plants" appeared as one of the later volumes of the collected works of Charles Darwin (Ref. 1). In his book, some 6oo pages long, Darwin described a fascinating behaviour pattern to which all rootlets, shoots, petioles, and leaf perform elliptical to circular motion. Why did Darwin begin - at the age of 71 and not in the best of health - 5 years before his death in 1882, a series of painstakingly planned experiments with about a dozen genera from completely different plant families on the circumnutation. His own discovery of the principle of evolution compelled him to do so, for according to this, it was impossible that the climbing plants should have developed into many groups if all plants did not possess some small capacity for movement.

PLANT MIMICRY

Sensitive plants were much discussed by philosophers, various theories being advanced to account for the rapid mimosa reaction. However only recently were investigators courageous enough to see a clear defence reaction in the fact that at the slightest touch the pairs of the pinnules folded together, first the pinnae and then the whole leaf pressing close to the rachis, and the whole plant pressing itself to the ground. The Mimosa plant becomes almost inconspicuous, exposing thorns on the stems. We agree with Hassenstein (Ref. 2) that this behaviour represents an excellent example of plant mimicry.

NASTIC MOVEMENTS

Movements, especially leaf movements, mostly fall into two categories: Rhythmic leaf movements in nyctinastic plants, and more rapid seismonastic movements, that occur in a limited number of nyctinastic species. Both movements were discussed already in the pre-Christian era, by Theophrast and Plinius T.E., Androsthenes, the admiral of Alexander the Great, described very early, 324/325 B.C., the plant sleep in the case of Tamarindus indica (Ref. 3). Analysis of leaf movements in the early eighteenth century provided the first clue that organisms have internal clocks (De Mairan 1729) (Ref. 4) - Zinn 1759) (Ref. 5) and subsequent studies from Pfeffer 1873 (Ref. 6) and Darwin 1881 (Ref. 1) are providing useful information of periodic nyctitropic movements of the pinnules of Leguminosae.

CHEMONASTIC MOVEMENTS

Likewise a hundred years ago, the physiological foundation of plant defence behaviour were discussed by Wilhelm Pfeffer in his general considerations "On the Nature of Stimulation Processes" (Ref. 7). In Pfeffer's opinion stimulation processes were at first only excitation processes and "accordingly, specifically for example, the sudden stimulation reactions - such as the closing of the leaves of the sensitive plant - are not of such general significance as the host of the slow and constantly occuring reactions and regulations". Regarding this, in Volume II of Pfeffer's Plant Physiology one can read that "it is presumed precise chemical stimuli that plays a prominent role in the autoregulatory guidance of inner activity and thus also of autonomous movement." Here reference is made to chemistry, the material basis of all stimulation processes which at every turn including the domain of plant defence mechanisms, confronts the natural products chemist with ever new perspectives. It is a stimulation but sometimes also a demanding task for analytical chemists to correlate observations with chemical structures revealing that Martin Lindauer had hit the nail right on the head in his book "The Biological Clock" (Ref. 8) when he said: "... that the basic requirement for all life is having the right substance in the right quantity, at the right place, at the right time."
To the chemist, the right substance means knowing the correct structure of an active principle which is only optimally effective when, as per Paracelsus, the dose is correct.The right place for a Leaf Movement Factor is the cell membrane where it must be present at the right time when the plant is about to fold its leaves to sleep.
In 1916 Ricca (Ref. 9) first postulated that this fascinating behaviour pattern, as Darwin had called it, must be due to a stimulant substance which was subsequently characterized by Fitting (Ref. 1o), Soltys and Umrath (Ref. 11) and a little later by Hesse (Ref. 12) as a hydroxycarboxylic acid with a molecular weight of between 35o and 5oo. A reducing substance was still suspected in 1957 (Ref. 13).

BIOASSAY

Wherever a movement factor is suspected, its aquous extract is prepared and tests are performed on it as such or after separation into components, making use of the rapid reactivity of M. pudica.In the bioassay in the climate chamber, a pinna of M. pudica is placed in a solution of the supposed active principles and is observed (Ref. 14). The movement factors are drawn up and cause each pair of the pinnules to fold up neatly one behind the other. The reaction behaviour induced by the chemonastic stimulus of a Mimosa crude extract can be demonstrated as a function of its concentration in a number of tests. Despite of individual variation which can always be observed in the bioassay, a clear decrease in the reaction time with declining concentrations can be seen. Forming the average values of the time of the first reaction movement and plotting them as a function of each concentration gives evidence for a hyperbola as shown in Fig. 1 (Ref. 15).

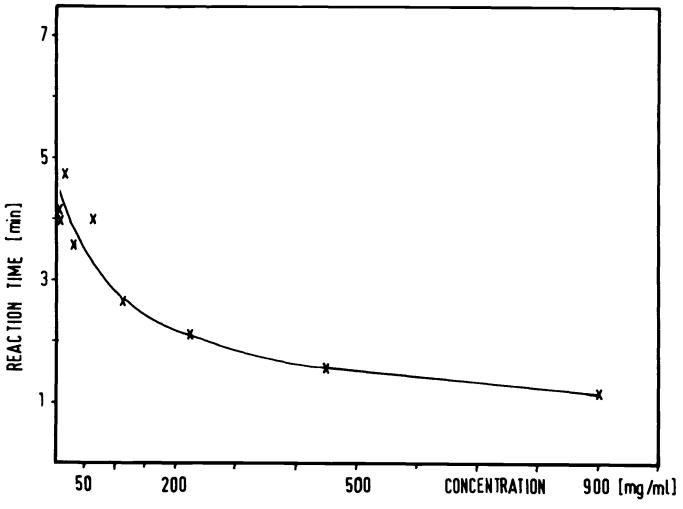


Fig. 1.Concentration/ average closing time diagram of M. pudica crude extract

The dependence of the reaction time upon the concentration of active compounds is used to find out the active fractions after a separation process.

AMINO ACIDS AS LEAF MOVEMENT FACTORS LMF (Ref. 16)

In order to elucidate the complex mode of the stimulus response in the Mimosaceae, we started with the study of free amino acids with different chromatographic methods. As some of these amino acids are active according to Fitting, we determined the amino acids quantitatively in fresh plants. In consequence of this analysis we measured the stimulating effect of some amino acids and mixtures of them. Remarkable is the excellent activity of mixtures of L-glutamic acid and ß-alanine. The activity of glutamic acid was characterized by periodical closures and opening movements in periods of 5 to 1o minutes. The test already indicates that the typical stimulus response of mimosa cannot be based on the amino acids. Amino acids are causing a bristly appearance of the leaves in the test. It is remarkable that the extracts free of amino acids causes a strictly behaviour of the pinnules which close exactly one after the other. Therefore we had to look for further Leaf Movement Factors not belonging to the amino acids.
First of all we isolated a restitution inhibitory compound. Restitution inhibitory means if the excised mimosa leaves are placed in the test solution, the pinnules are prevented from opening. Studying physical constants and spectral data (MS, IR and NMR) led to the identification of the compound as D-pinitol (1)(Ref. 17). Acetylation of pinitol strikingly destroys its

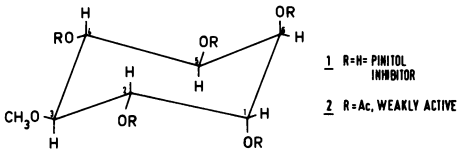


Fig. 2. D-pinitol

mimosa restitution inhibitory power and surprisingly turns it into a weak mimosa active compound (2). Further compounds like myo-inositol and methyl D-glucoside, closely related to pinitol,did not show any inhibition on the recovery of the closed mimosa leaves indicating that this particular activity of pinitol is quite specific.

ORGANIC ANIONS AS LEAF MOVEMENT FACTORS (LMF) OF A STIMULATION CHAIN

The results of an electrodialysis gave the first hint that M-LMF might be an anion (Ref. 18). Then we obtained a highly active fraction along a large separation process (Ref. 19).
Hydrolysis of the M-LMF 1 resulted in a strong reducing acid and a sugar. The ^{1}H-NMR-spectrum was much more informative. In the low field region one recognizes the resonances of an aromatic system. One can detect an anomeric proton and a ß-anomeric proton. This is a hint that the sugar moity is linked ß to the aromatic system. In the region from 3,4 to 4,2 ppm are the eleven ring protons of the oligosaccharide. As there will be always lack of substance for an intensive NMR investigation of the M-LMF 1, only an ultramicro sugar sequence analysis with the aid of MS helped in the structure elucidation. The fact that the first leaf movement factor from M. pudica is the gentisic acid glucoapioside was first fully recognized in 1978, and the last structure problems were solved by coanalytical investigation. Probably the most important result was that K-LMF 1 from Acacia karroo must be very similar to the M-LMF 1 of Mimosa pudica (Ref. 2o). We were then able to establish the identity of K-LMF 1 with M-LMF 1 by means of ^{13}C-NMR-spectroscopy.
The structure elucidation has now been completed by the total synthesis of M-LMF 1 (Ref. 21). Alkaline acetylation of gentisic acid (3) gave 5-acetyl-gentisic (4) acid which after reaction with CH_2N_2 gave 5-acetyl-gentisic acid methyl ester (5). Di-isopropyliden-glucofuranose (8)from glucose and

acetone, was converted to 3-O-benzylglucose (9) by two steps:1. benzylation of the free hydroxy group and 2. acid catalysed cleavage of the ketal group. Acetylation followed by reaction with HBr/HOAc yielded the corresponding protected halogenose (10). Koenigs-Knorr-reaction of compound (7) and (10) gave, after several modifications the specifically protected aryl-monoglucoside (12).

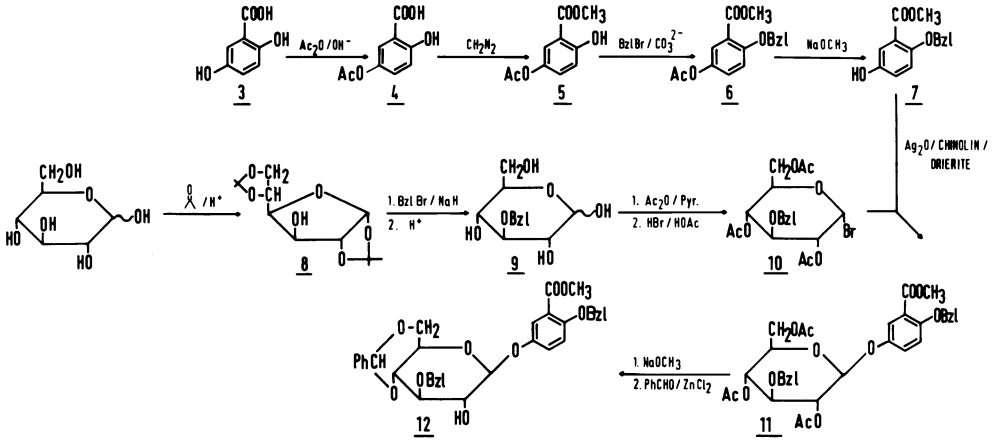


Fig. 3. Synthetic pathway of M-LMF 1 (part 1)

Di-isopropyliden-mannofuranose (13) was converted by reaction with formalin into the 2-hydroxy-methylderivative (14). Partial cleavage of one ketal group, reduction with $NaBH_4$ and double glycol cleavage with $NaJO_4$ resulted in 2,3-isopropyliden-apiose (15). After alkaline acetylation and removal

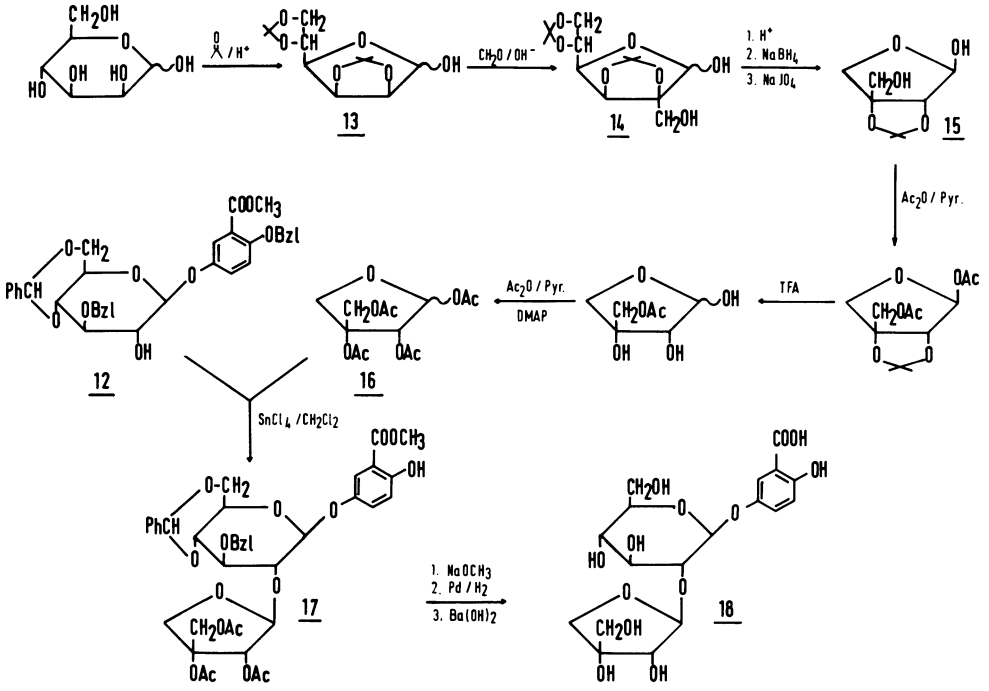


Fig. 4. Synthetic pathway of M-LMF 1 (part 2)

of the ketal groups with TFA, further acetylation by Ac_2O/Pyr/DMAP led to the peracetyl-apiose (16). Glycosidation with $SnCl_4$ gave the substituted aryl-disaccharide (17) which was converted by removal of all protective groups into the free synthetic factor M-LMF 1 (18). All spectral data known from the M-LMF 1 are confirmed by a spectroscopic analysis.
We were not suprised about the negative results from the bioassay with the synthetic compound. After all, the M-LMF 1 is only a member of a stimulation chain that definitely also comprises amino acids and possibly even an inhibitor, D-pinitol (1).

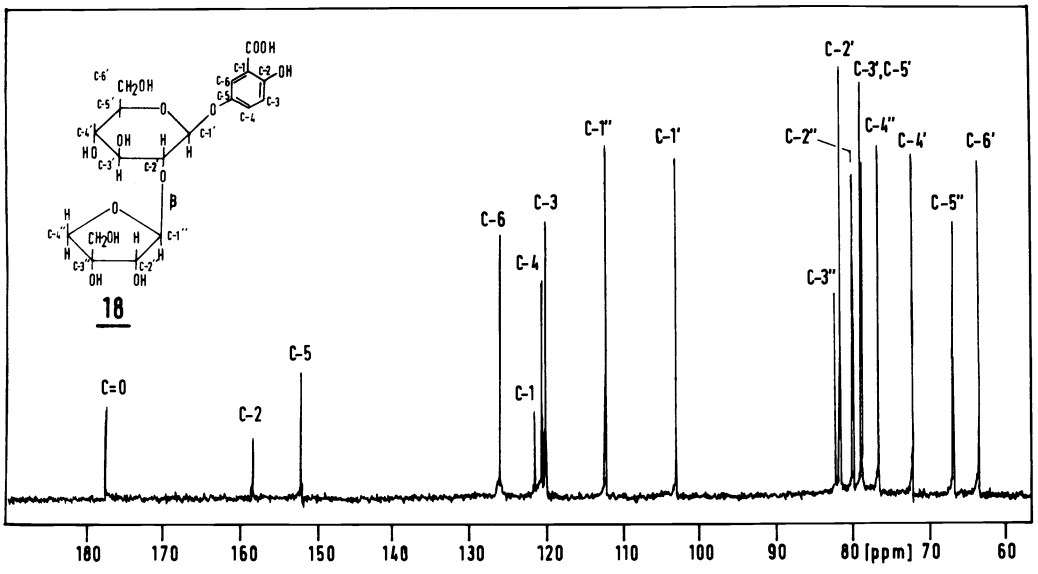


Fig. 5. 75.46 MHz ^{13}C-NMR-spectrum of M-LMF 1 in D_2O
Technique: wide-band decoupling, internal reference C-3

In any event, it seemed appropiate to look for further members of this chain, such were found in a highly enriched active fraction. Guided by a bioassay, from 4,5 kg dry extract of M. pudica some fractions free of amino acids were isolated by a MeOH-precipitation, ion-exchange chromatography on Lewatit TSW 4o and filtration on polyacrylamid. These fractions proved to be active in the bioassay. They could be further purified on Sephadex LH 2O so it was possible to isolate two new LMF by evaporation and precipitation at low temperature (Ref. 22).
Preliminary tests indicated cyclic nucleotides which were detected in plant tissue only in recent years. That is why an especially accurate spectroscopic analysis was advisible. At first two absorption maxima at 252 and 275 were registered for the M-LMF 2 which are typical for derivatives of Guanosine. A positive Cotton-effect was in accordance with that of reference substances.
But only after intensive studies by IR-spectroscopy and PFT-^{1}H-NMR-spectroscopy with an imponderable amount of the natural product 2'.3' cyclic guanosinemonophosphate (19) was reliably demonstrated.
After the identification of M-LMF 2 and other components in the active fraction of the EtOH/H_2O-partition a further compound namely 2'.3' cyclic adenosinemonophosphate (2o) could be identified by comparing the 3oo MHz-^{1}H-NMR spectra of a couple of reference substances.

Although M-LMF 2 and 3 are inactive in pure form in the above described bioassay, they are definitely also important components of the whole active principle complex. One could almost suppose that these LMF are stimulus-potentiating factors, just as the 6-hydroxy-purine-5'-mononucleotide was identified as a long-sought flavor potentiator (Ref. 23).

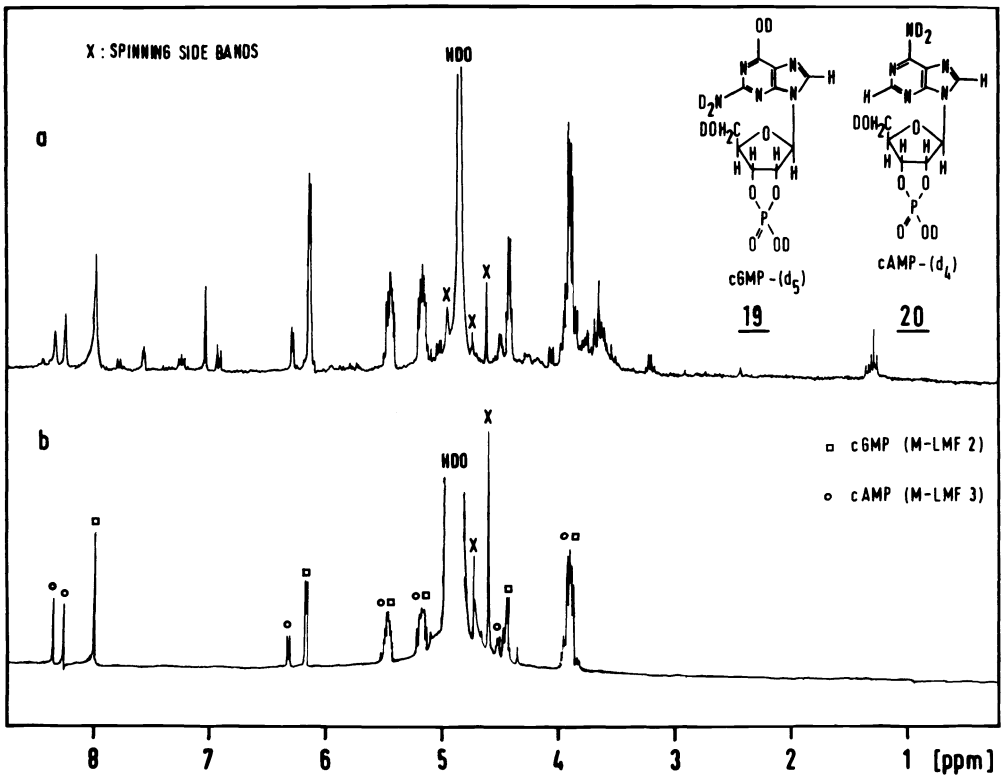


Fig. 6. 3oo MHz-^{1}H-NMR-spectra of M-LMF 2 and M-LMF 3 in D_2O
a: in natural product, 1oo4 scans
b: in synthetical mixture, 126o scans

Comparison of the 3oo MHz-^{1}H-NMR-spectra of the natural product with the spectrum of authentic material (Fig.6) revealed the presence of minor components which are characterized by signals at about 7 ppm and 3,6 ppm. Earlier investigations have shown that these compounds may be present in higher concentrations in an extract of Acacia karroo.

PERIODIC LEAF MOVEMENT FACTORS (PLMF) OF ACACIA KARROO

Acacia karroo is not sensitive but it too folds up its pinnae at night, then it looks like a stimulated mimosa.
It has been found out that for optimal activity still other glycosides as those of phenolic carboxylic acids, already mentioned, must be present. For the isolation and purification of the hitherto unknown LMF, a dried water extract of A. karroo was dissolved in water, a methanol precipitation was carried out and the filtrate was subjected to liquid extraction with n-butanol and water. The concentrated water extract was chromatographed on polyacrylamid gel P-2 after it has been filtrated through the same material.
Separation from amino acids was achieved by filtration with Sephadex G-1o. The final separation was accomplished by distribution chromatography on Sephadex LH 2o. The active material was eluted with the last fraction (Fig. 7). In this fraction we found again gentisic acid glucoapioside (18) as K-LMF 1 and the cyclic nucleotides 2'.3'-guanosine cyclomonophosphate (19) and 2'.3'-adenosine cyclomonophosphate (2o) as K-LMF 2 and K-LMF 3 (Fig. 8). The cyclic nucleotides were characterized by UV-,IR-,^{1}H-NMR-and ^{13}C-NMR-spectra.

Two further nucleotides with nearly the same chromatographic behaviour on LH 2o as the K-LMF 3 were identified by UV-, IR-, ^{1}H-NMR-spectroscopy as 3'-guanosine monophosphate and 3'-adenosine monophosphate. These open nucleotides are most probably no artefacts. Separation of all four nucleotides can be achieved by HPLC on Nucleosil C-18 (Table 1).

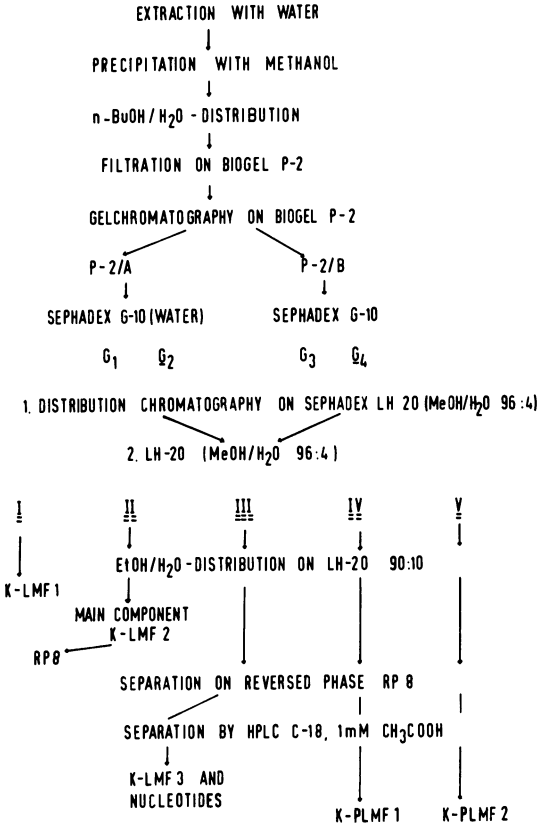


Fig. 7. Separation Scheme of K-LMF and PLMF

TABLE 1. Comparison of retention time (HPLC) of nucleotides from active fractions of Mimosa pudica and Acacia karroo

NUCLEOTIDES	AUTHENTIC SUBSTANCES t_r (MIN)	M. PUDICA	A. KARROO
2'.3'-cAMP	10,3	10,4	10,3
3'-AMP	8,6	8,7	8,7
2'.3'-c GMP	4,25	4,3	4,3
3'-GMP	3,6	3,65	3,6
25 cm NUCLEOSIL C-18, 5 µ, 8mm, 1 mM HOAc, 2400 PSI, 5 ml/min, UV: 254nm.			

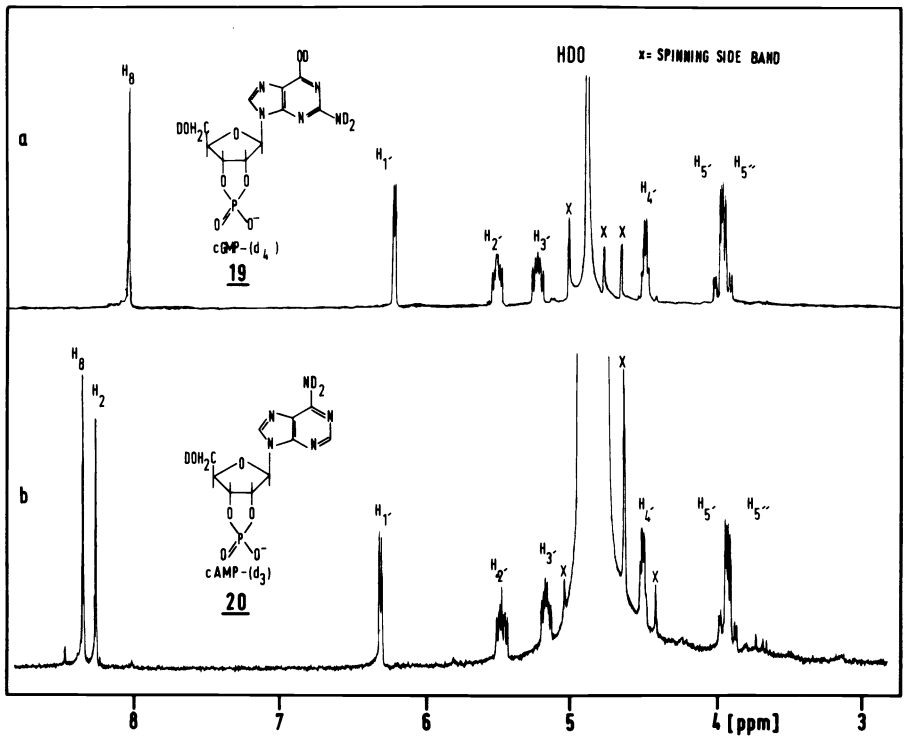


Fig. 8. 300 MHz ^{1}H-NMR-spectra of natural products of A. karroo in D_2O
a: K-LMF 2, 1200 scans b: K-LMF 3, 1830 scans

From the last highly active fractions of LH 2o chromatography we isolated by HPLC on reversed phase C-18 two new leaf movement factors K-PLMF 1 and K-PLMF 2.

In the preliminary spectroscopic tests the UV-spectrum displayed two absorption maxima, one at 245 nm and one at 285 nm which indicate a derivative of gallic acid. The mass spectrum (Table 2) with ions at m/z 17o, 153, 126, 125, 1o7 and 79 confirmed this suggestion (Ref. 24).

$\frac{m}{z}$	ACCURATE MASS	ELEMENTAL COMPOSITION	DEVIATION (mmu)
64	63,9609	SO_2	+ 1,0
79	79,0178	C_5H_3O	+ 0,6
80	80,0266	C_5H_4O	- 0,4
95	95,0105	$C_5H_3O_2$	+ 2,8
97	97,0276	$C_5H_5O_2$	+ 1,3
107	107,0131	$C_6H_3O_2$	+ 0,2
108	108,0219	$C_6H_4O_2$	- 0,8
110	110,0371	$C_6H_6O_2$	- 0,3
125	125,0242	$C_6H_5O_3$	- 0,3
126	126,0337	$C_6H_6O_3$	- 2,0
153	153,0199	$C_7H_5O_4$	- 1,1
170	170,0197	$C_7H_6O_5$	+ 1,8

TABLE 2. Elemental composition of fragments of K-PLMF 1 measured with a double focusing mass spectrometer ZAB 2 F using the direct inlet system, sample temperature was 3oo° C.

Of special importance was the appearance of m/z 63,96o9 which gave evidence for a SO_2 group. We also found in the IR-spectrum a S=O stretching vibration at 1248 cm^{-1} and an absorption at 818 cm^{-1} which is typical for sulfates of primary alcohols (Ref. 25).
Actually the sulfate could be precipitated as Barium-sulfate which is proved by the two identical Debye-Scherrer-diagrams.
In the ^{1}H-NMR-spectrum (Fig.9) of the K-PLMF 1 of A. karroo the signal at δ = 7.12 corresponds to the resonance of the two ring protons H-2 and

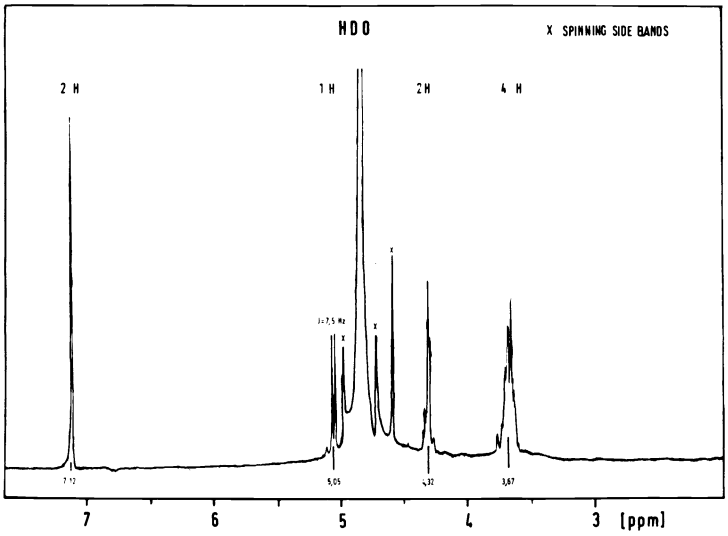


Fig. 9. 3oo MHz-^{1}H-NMR-spectrum of K-PLMF 1 in D_2O, 21o scans

H-6 of gallic acid. The relation of the intensity of the aromatic and aliphatic protons 2:7 suggests a hexose which was identified as glucose by GC-MS analysis of the trimethylsilylated products of the hydrolysis. With this in mind the signal at δ = 5,o5 with a coupling constant of J = 7,5 Hz can be identified as the resonance of the anomeric proton of a D-glucopyranose with ß-linkage. Since we found only one singulet at 7.12 ppm, we had to assume a gallic acid with symmetric substitution. An ester linkage was improbable because the two ring protons H-2 and H-6 of gallic acid showed a chemical shift of 7,3 ppm. Therefore linkage via the OH-group at C-4 was likely which was proved by IR-, ^{13}C-NMR and MS-spectra. The proton resonances of glucose with ß-linkage with different phenolic carbonic acids are very similar. Besides the resonance of the anomeric proton we find the chemical shifts not higher than 4 ppm. Since we find a signal at 4,32 ppm in the proton resonance spectrum of the K-PLMF 1 with an intensity of two protons, we have to assume a substitution at the CH_2OH-group of the glucose. Comparison with data from the literature and measurements with 5'-adenosine monosulfate make the position at C-6 of the glucose for the sulfate group very likely.
We observed in the mass spectrum (Fig. 1o) of the trimethyl-silylated active compound from A. karroo which was carried out with accurate mass measurement, the displacement of covalently bonded sulfate by the silylation reagent. An ion m/z 227.229 was found with an elemental composition which is consistent to trimethylsilylated sulfate minus a methyl group.

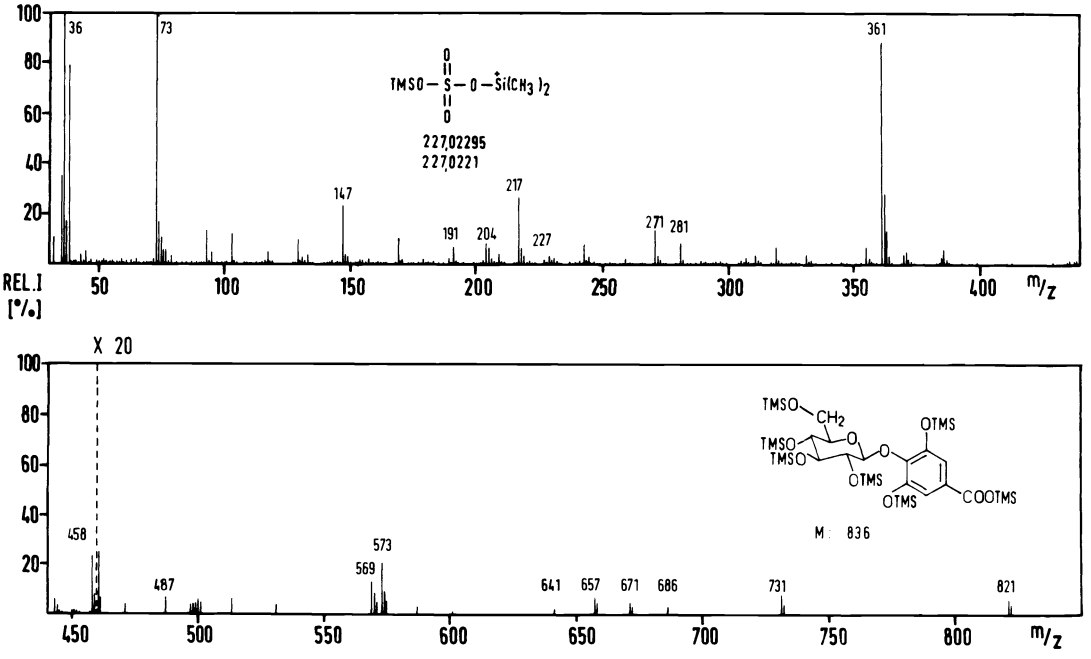


Fig. 1o. EI mass spectrum of silylated K-PLMF 1 by direct inlet

In this connection ion m/z 573.2371 ($C_{24}H_{45}O_7Si_4$) is of special interest. Its genesis can be explained by loss of CO_2, CH_3, 2 x OTMS and C_2H_2 from the molecular ion of trimethylsilylated gallic acid glucoside. This fragmentation substantiated the assumption of a free carboxyl group. Ion m/z 458 has the composition of the persilylated gallic acid, it is originated by breakage of the glucoside bond and migration of a trimethylsilyl group.

Final proof for covalently bonded sulfate came from the negative ion fast atomic bombardement (FAB) mass spectrum (Fig. 11). We used a beam of highly accelerated Xenon atoms as agent for sputtering ionization and embedded the compound in a matrix of glycerol. The spectrum exhibited the key fragments m/z 411, 241, 169 and 97 which correspond to the ions of gallic acid glucoside sulfate minus one, to gallic acid glucoside sulfate minus gallic acid, to gallic acid minus one and to HSO_4 (Ref. 26).

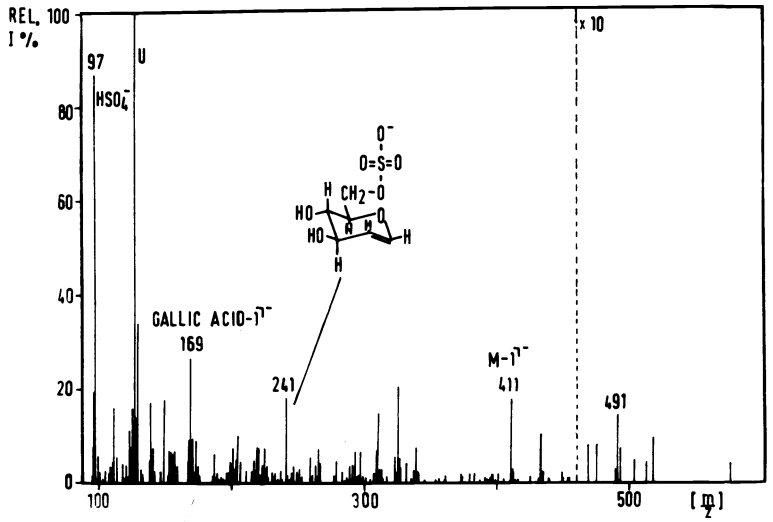


Fig. 11. Negative ion FAB mass spectrum of K-PLMF 1

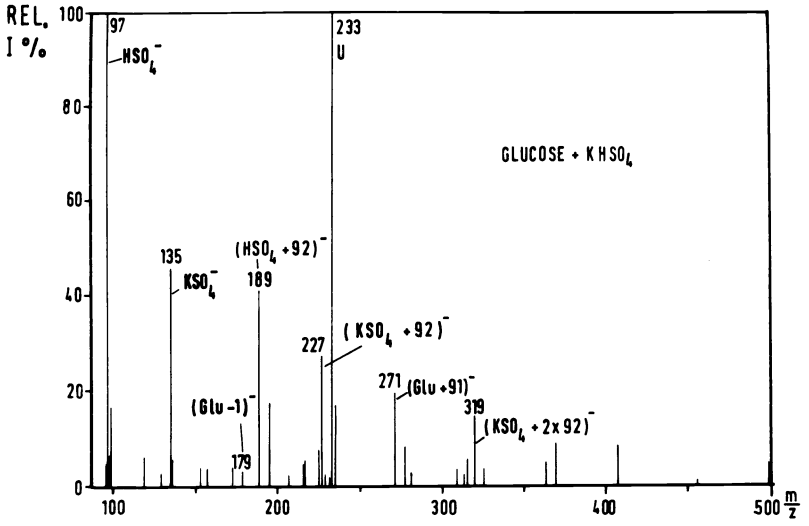


Fig. 12. FAB spectrum of a mixture of glucose and $KHSO_4$ (negative ions)

We were able to demonstrate that the incorporation of inorganic sulfate during the time the spectrum is shot is very unlikely. Since the negative ion FAB spectrum of a mixture of glucose and hydrogen sulfate (Fig. 12) shows no addition of sulfate, but only the quasi molecular ions of glucose and glucose glycerol adducts and is dominated by the ions derived from inorganic sulfate while the negative ion FAB spectrum of glucose sulfate is absolutely dominated by the quasi molecular ion of glucose sulfate m/z 259 (M-1).

The proposed gallic acid 4-glucoside-6'-sulfate (21) was confirmed by the ^{13}C-NMR spectrum (Fig. 13). We assigned the signals above 11o ppm to the ^{13}C-atoms of gallic acid. The signal at δ = 1o6,7 verifies the ß-glucosidic linkage of C-1' of the glucopyranose. In the narrow region between 62 and 64 ppm the ^{13}C-6 signal of the hydroxymethyl group of hexoses appears. A shift of about 6 ppm to lower field is anticipated in case of esterification with sulfate. We therefore expected the corresponding signal of the K-PLMF 1 between 68 and 7o ppm. In agreement with this assumption we found

in the Off-Resonance spectrum a triplet at δ = 69,4. A study of the influence of the sulfate substituent on the ^{13}C-resonance led to the conclusion, that the ^{13}C-atoms in ß-position suffer a shift to high field of about 2 ppm.

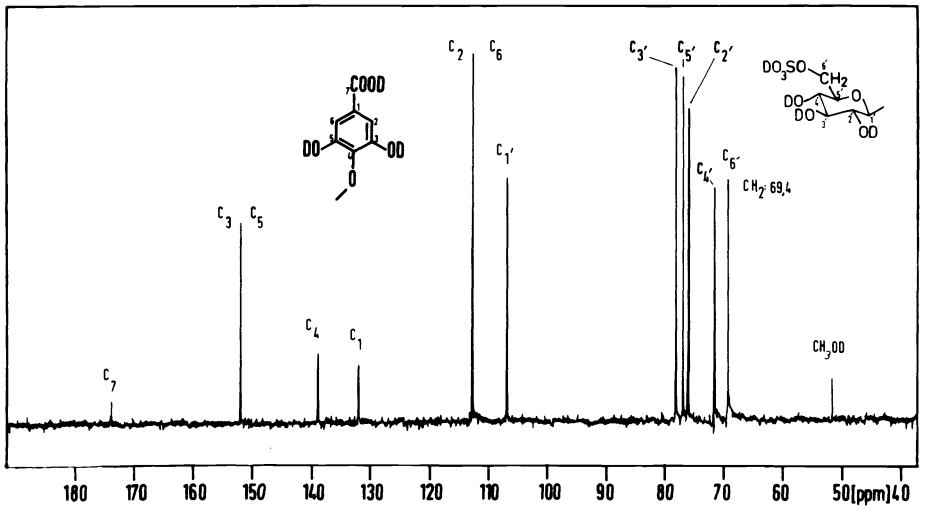
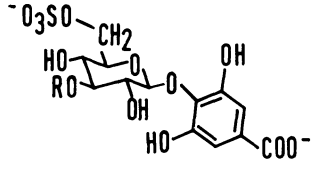
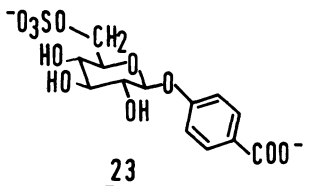


Fig. 13. 75,46 MHz ^{13}C-NMR-spectrum of K-PLMF 1 in D_2O (48o ooo scans)
Technique: wide-band decoupling, reference signal CH_3OD

We expected therefore the resonance of the ^{13}C-3'-atom of the K-PLMF 1 at a lower field than the signal of the ^{13}C-5'-atom. The exact assignment of the signals of the ^{13}C-2', ^{13}C-3', ^{13}C-4' and ^{13}C-5' atoms was easily possible with the aid of correlation diagrams.

Based on spectroscopic investigations, it was possible to assign structure (21) for the newly found K-PLMF 1 and a very similar structure (22) for the K-PLMF 2.





23

		ACTIVITY
21	R= H K-PLMF 1	10^{-4} g/l
22	R= SO_3^- K-PLMF 2	10^{-4} g/l

ACTIVITY

7×10^{-2} g/l

They are single highly active movement compounds which cause a specific mimosa reaction in a concentration as low as $1o^{-7}$ mol/l. From great interest for us was the fact that a synthetic p-Hydroxy-benzoic acid glucoside-6'-sulfate (23) showed an activity of $1o^{-4}$ mol/l (Ref. 27).
The concentration/activity diagrams of the Movement Factors are similar to the diagram of a crude extract from M. pudica.

Final proof for the proposed structure of K-PLMF 1 came from the synthesis (Ref. 28). The 3.5-diacetyl gallic acid methylester (24) was converted by Koenigs-Knorr reaction to the peracetyl-gallic acid 4-O-glucoside methyl-ester (25). After two steps of saponification we got the K-PLMF 1 (21) by reaction with a pyridine-sulfate-trioxide complex (Fig. 14).

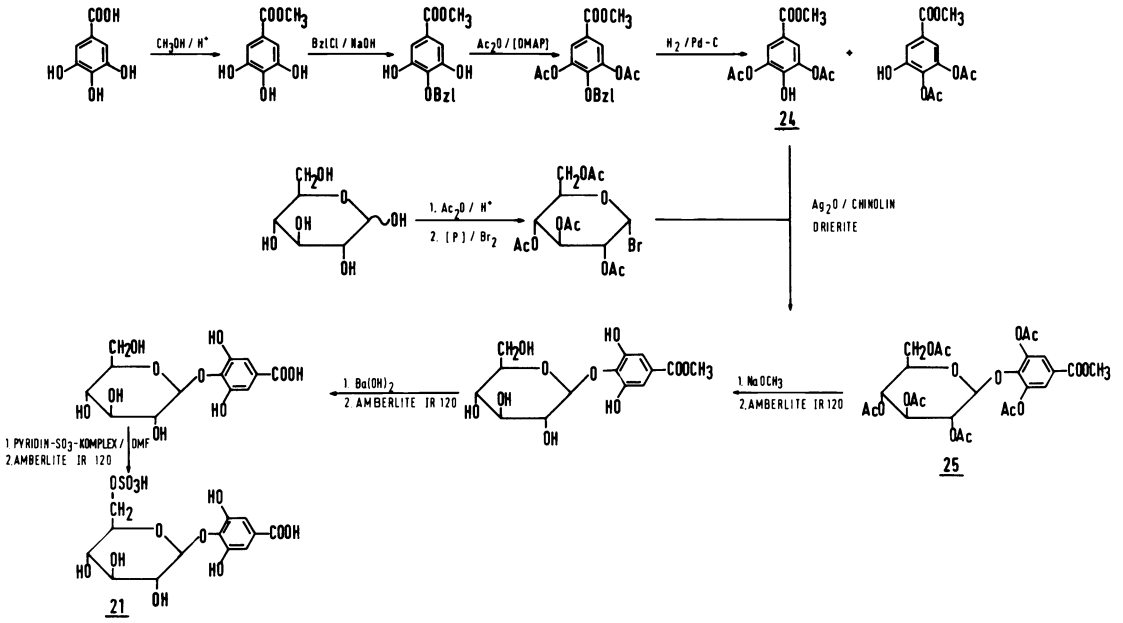


Fig. 14. Synthetic pathway of K-PLMF 1

The ^{1}H-NMR-spectra of the natural and synthetic compounds (Fig. 15) are identical, and the concentration/activity diagram of a synthetic sample is similar to the Mimosa extract (Fig. 19).

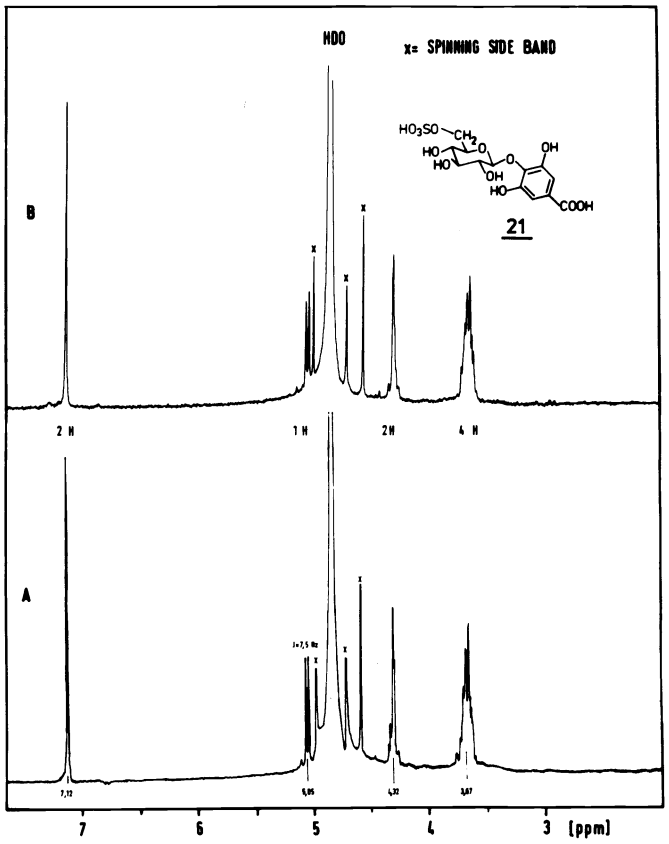


Fig. 15. 3oo MHz-^{1}H-NMR-spectra of K-PLMF 1 in D_2O
A: natural product, 21o scans
B: synthetic compound, 28o scans

Such plots are informative for a special kind of Leaf Movement Factor as the radioactive period for a radioactive element. With other words, we have found the chemical code for nyctinastic and seismonastic movement as well, and perhaps for the inner clock of plants.

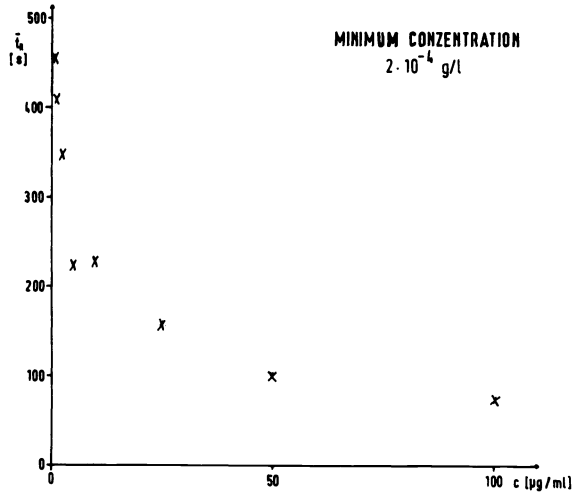


Fig. 16. Relation between reaction time and concentration of synthetic K-PLMF 1

Again the questions arises: Are the newly discovered movement compounds from Acacia karroo the active principle of nyctinasty or the chemical basis of other nasties as well? A comparative study of the chemistry of the several species of leguminoseae and oxalidaceae has elucidated some basic scientific truth for the Leaf Movement Factors discussed. Therefore we gave them the name Nyctinastenes (Table 3).

PLANT	t[s]	K-PLMF1	LOCATION
MIMOSA PUDICA	25	+	HEIDELBERG
ACACIA KARROO	30	+	SOUTH AFRICA
ACACIA DEALBATA	40	+	SOUTHERN FRANCE
ALBIZIA JULIBRISSIN	50	+	HEIDELBERG
GLEDITSIA TRIACANTHOS	60	+	HEIDELBERG
ROBINIA PSEUDACACIA	60	–	HEIDELBERG
GLYCINE MAX.	120	–	HEIDELBERG
OXALIS ACETOSELLA	50	?	HEIDELBERG
OXALIS STRICTA	40	+	HEIDELBERG
OXALIS DEPPEI	40	?	HEIDELBERG
ABUTILON GRANDIFLORUM	25	?	TENERIFE

TABLE 3. Plants whose extracts are "active" in the bioassay (Ref. 15, 29, 3o, 31, 32, 33, 34, 35, 36)

In our future research regarding the nyctinastenes of higher plants we are especially interested in Abutilon grandiflorum (Ref. 36), a representative member of Malvaciae family; further matters of interest are Oxalis deppei pf. (Ref. 34) and Albizzia julibrissin (Ref. 3o), a Mimosaceae. The leaves of this plants belonging to different families exhibit nyctinastic, photonastic and thermonastic as well as weak seismonastic movements.

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