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(54)【発明の名称】１級アルコールの選択的酸化方法と新規な炭水化物アルデヒド

(57)【要約】

ニトロキシル化合物を酸化可能である酵素化合物の存在下で酸化することにより得られる、触媒量のニトロソニウム化合物を用いて、１級アルコール、特に炭水化物が酸化されるアルデヒド、及び／またはカルボン酸を製造する方法が記述されている。更に記述されているのは、２５個のモノサッカライド単位当たり及び分子当たり１個のカルボアルデヒド基を持つ少なくとも１個の環状モノサッカライド鎖基を含む、酸化された炭水化物である。

【特許請求の範囲】

【請求項１】

ニトロキシル化合物が酸化可能である酵素の存在下で及び／または金属錯体の存在下で酸化されることを特徴とする、ニトロキシル化合物を酸化剤で酸化することによりニトロソニウムイオンを製造する方法

【請求項２】

該ニトロキシル化合物がジーｔーニトロキシル化合物、特に２，２，６，６－テトラメチルピペリジン－１－オキシル（ＴＥＭＰＯ）である請求項１に記載の方法。

【請求項３】

該酸化可能である酵素が酸化還元酵素である請求項１または２に記載の方法。

【請求項４】

該酵素がペルオキシダーゼ、特にホースラデッシュペルオキシダーゼ、大豆ペルオキシダーゼ、リグニンペルオキシダーゼまたは骨髄－あるいはラクトーペルオキシダーゼであり、該酸化剤が過酸化水素である請求項３に記載の方法。

【請求項５】

該酵素がポリフェノールペルオキシダーゼまたはラッカーゼであり、該酸化剤が酸素である請求項３に記載の方法。

【請求項６】

該酵素が金属化合物の存在下でヒドロラーゼ、特にフィターゼまたはリパーゼである請求項１または２に記載の方法。

【請求項７】

ニトロソニウムイオンが請求項１－６のいずれか一つの方法により製造されることを特徴とする、触媒としてニトロソニウムイオンにより１級アルコールを酸化する方法。

【請求項８】

該１級アルコールが炭水化物、特にα－グルカンまたはフルクタンまたはこれらの誘導体に含まれる請求項７に記載の方法。

【請求項９】

２５個のモノサッカライド単位当たりそして平均分子当たり１個のカルボアルデヒド基を持つ少なくとも１個の環状モノサッカライド鎖基を含むカルボニル基含有炭水化物が製造される請求項８に記載の方法。

【請求項１０】

該炭水化物がヒドロキシアルキル化された炭水化物またはグリコシドまたはグリコン酸である請求項８または９に記載の方法。

【請求項１１】

該１級アルコールがステロイド化合物に含まれる請求項１－７のいずれか一つに記載の方法。

【請求項１２】

木綿繊維が請求項１－６のいずれか一つの方法により製造されるニトロソニウムイオンにより処理されることを特徴とする、テキスタイル繊維を処理して、アルデヒド基を導入する方法。

【請求項１３】

酸化された炭水化物であって、該炭水化物が２５個のモノサッカライド単位当たりそして平均分子当たり１個のカルボアルデヒド基を持つ少なくとも１個の環状モノサッカライド鎖基またはこれらの化学的誘導体を含む、グルカン、マンナン、ガラクタン、フルクタン、及びキチンタイプのジサッカライド、オリゴサッカライド及びポリサッカライド、及び炭水化物グリコシドから選ばれるもの。

【請求項１４】

平均分子当たり少なくとも５個のモノサッカライド単位を含む請求項１３に記載の酸化された炭水化物。

【請求項１５】

５０個のモノサッカライド単位当たりそして平均分子当たり１個のカルボアルデヒド基を持つ少なくとも１から５０個の環状モノサッカライド鎖基を含む請求項１３または１４に記載の酸化された炭水化物。

【請求項１６】

該誘導体において、少なくとも一部のカルボアルデヒド基が式－ＣＨ＝Ｎ－Ｒまたは－ＣＨ$_2$－ＮＨＲの基（式中、Ｒは水素、ヒドロキシル、アミノ、または基Ｒ^1、ＯＲ^{1}またはＮＨＲ^1（ここで、Ｒ^{1}はＣ$_1$－Ｃ$_{20}$アルキル、Ｃ$_1$－Ｃ$_{20}$アシル、炭水化物残基、また炭水化物残基と結合した、あるいは結合可能である基である）に変換された請求項１３－１５のいずれか一つに記載の炭水化物誘導体。

【請求項１７】

誘導体において、少なくとも一部のカルボアルデヒド基が式－ＣＨ（ＯＲ^3）－Ｏ－ＣＨ$_2$－ＣＯＯＲ^{2}または－ＣＨ（－Ｏ－ＣＨ$_2$－ＣＯＯＲ^2）$_2$の基（式中、Ｒ^{2}は水素、金属カチオンまたは場合によっては置換アンモニウム基であり、そしてＲ^{3}は水素または炭水化物の脱水素化されたヒドロキシル基の酸素原子への直接結合である）
に変換された請求項１３－１５のいずれか一つに記載の炭水化物誘導体。

【請求項１８】

カルボキシル及び／またはカルボキシメチル基を更に含む請求項１３－１７のいずれか一つに記載の炭水化物。

【発明の詳細な説明】

【０００１】

本発明は、ニトロキシルラジカル、特に２，２，６，６－テトラメチルピペリジン－１－オキシル（ＴＥＭＰＯ）の酸化によるニトロソニウムイオン（オキソアンモニウムイオン）の生成に関する。このニトロソニウムイオンは、１級アルコールをアルデヒドに選択的酸化するための触媒的酸化剤として使用され得る。

【０００２】

化学的手段によりＴＥＭＰＯを再酸化するこのような方法は、ＤｅＮｏｏｙ，Ｓｙｎｔｈｅｓｉｓ，１９９６、１１５３－１１７４によるレビュー及びＷＯ９５／０７３０３から知られる。

【０００３】

塩素ベースの酸化剤を使用せずに、そして最終酸化剤として過酸化水素または酸素を使用して、アルコール官能基、特に１級アルコール官能基の酸化を行うことができることが本発明により見出された。本発明による酸化は、酵素及び／または金属錯体を用いて行われる。驚くべきことには、１級アルコールについて行われる場合には、この酸化は、結果として、所望ならば適切な条件を用いてカルボキシル基への実質的に更なる酸化なしでアルデヒドを生成せしめる。このアルデヒドは、（ヘミ）アセタール形及び関連する構造で存在してよい。本発明の酸化方法の適用を使用して、２級アルコール、特に炭水化物をケト誘導体に酸化することができる。本発明の方法は、添付のクレームの特徴を記述する要点により更に規定される。

【０００４】

非事前公開の国際特許出願ＷＯ９９／２３１１７及びＷＯ９９／２３２４０は、ラッカーゼ等の酸化性酵素を酸素及びＴＥＭＰＯの媒介と共に使用して、それぞれセルロースまたは澱粉を酸化することを記述している。セルロースのラッカーゼ／ＴＥＭＰＯ酸化は、結果として低レベルの未特定カルボキシル及びカルボニル基の存在を生じせしめ、一方、澱粉のラッカーゼ／ＴＥＭＰＯ酸化は、１００個のグルコース単位当たり１個のカルボキシル基と３個のアルデヒド基を持つ生成物を生じると報告されている。アルデヒド含量を定量する方法は示されていない。

【０００５】

次の説明においては、簡便のために、ＴＥＭＰＯのみを引用するが、他の好適なニトロキシル、すなわち２，２，５，５－テトラメチルピロリジン－Ｎ－オキシル（ＰＲＯＸＹＬ）、４－ヒドロキシ－ＴＥＭＰＯ、４－アセトアミド－ＴＥＭＰＯ及びこれらの誘導体等

のα－水素原子を欠く有機ニトロキシル化合物とＷＯ９５／０７３０３に記述されているものをＴＥＭＰＯに置き換えることができると理解されるべきである。これらのジ－ｔ－アルキルニトロキシルは、特に酸化対象でない２級アルコール官能基の存在下で１級アルコールをアルデヒド官能基に選択的に酸化するのに特に好適である。４，４－ジメチルオキサゾリジン－Ｎ－オキシル（ＤＯＸＹＬ）等の立体障害の少ないニトロキシルは、例えば核のセルロースまたはケト澱粉の製造において２級アルコールをケト官能基に優先的に酸化するのに好適である。活性な酸化性種は、対応するヒドロキシルアミンとニトロキシルラジカルの酸化により系内で生成するニトロソニウムイオン（オキソアンモニウムイオン＞N^+＝Ｏ）である。所望する場合には、ニトロソニウムイオンの生成を第１に、そしてアルコール官能基の酸化を第２というように、この反応を２ステップで行うことができる。

【０００６】

触媒量のニトロキシルは、好ましくは１級アルコール基準で０．１－２５重量％、あるいは１級アルコールに対して０．１－２５モル％である。このニトロキシルは、また、例えば４－ヒドロキシ－ＴＥＭＰＯのヒドロキシル基を好適なキャリアに結合することにより、あるいは－［（CH_3）$_2$Ｃ－ＮＯ－Ｃ（CH_3）$_2$－Ａ］$_n$－（式中、Ａはアルキレン基及び／またはヘテロ原子であってよく、ｎは例えば１０から数１００迄の数である）等のポリマーニトロキシルの形で固定化されてもよい。

【０００７】

本発明の方法は、最初に、１級アルコールの対応するアルデヒドへの酸化に使用され得る。必要ならば、次亜塩素酸塩、塩素酸塩、過酸化水素等の既知の酸化剤を使用することにより、あるいは上昇した温度、例えば４０－８０℃等の更に苛酷な条件下で、あるいは反応条件への長期曝露の間にＴＥＭＰＯ－媒介の酸化を使用することにより、この１級の生成物を対応するカルボン酸に更に酸化することができる。あるいは、比較的低いｐＨ（例えば、ｐＨ３－７）を使用することにより、酸化剤をコントロールして添加することにより、酸素濃度を低下することにより、あるいは最初にニトロソニウムイオン溶液を製造する（２ステッププロセス）ことにより、アルデヒド／カルボン酸比を増加することができる。

【０００８】

本方法は、１，６－オクタンジオール、１，９－オクタデカンジオール、ステロイドホルモン、シュガーアルコール、グリコシド（風味前駆体）等の、１級アルコールに加えて２級アルコール官能基を持つアルコール、特に１級アルコール官能基を持つ炭水化物における１級ヒドロキシル基の選択的酸化に特に有利である。この炭水化物は、グルコース、フルクトース等のモノサッカライド、スクロース、マルトース、ラクトース等のジサッカライド、オリゴサッカライド、及びポリサッカライドであってよい。このオリゴ－及びポリサッカライドは、いかなるタイプであってもよく、例えば、澱粉、澱粉成分（すなわち、アミロース、アミロペクチン、デキストリン）等のグルカン、プルラン（α－１，４－α－１，４－α－１，６－グルカン）、セルロース（特に非－木材）、キチン、リケニンなど、イヌリン及びレバン等のフルノフルクタン、ガラクタン、アラビノガラクタン、フラノイドペントサン（キシラン）、（ガラクト）マンナン（グアー、イナゴマメガム）、細菌型エクソポリサッカライド（ＥＰＳ）など及び加水分解物等のこのような炭水化物の誘導体である。これらのオリゴ－及びポリサッカライドは、ヘテロサッカライド、すなわち、例えば、キサンタン及び藻から誘導される炭水化物におけるように、これらの異なる単位自身がウロン酸単位等の１級ヒドロキシル基を持たないとしても、異なる構造単位を持つものを含む。本発明により酸化を受ける炭水化物は、グリコシドと他の保護された炭水化物を含む。更なる例は、デルタ－ラクトン等のグリコン酸であり、これは、グリカル酸などに酸化され得る。

【０００９】

本方法による酸化に好適な化合物の明確な群は、ヒドロキシプロピルセルロース、ヒドロキシエチル澱粉またはヒドロキシエチルイヌリン等のヒドロキシアルキル化炭水化物から

なり、これらは、結果としてホルミルアルキル炭水化物を製造する代替法を与える。少なくとも一部の（６－）ヒドロキシメチル基が損なわれないままの他の好適な炭水化物基質は、例えば（２－及び３－）カルボキシメチル炭水化物を含む。

【００１０】

１級ヒドロキシル基を含む炭水化物の酸化は、結果としてアルデヒドを含む対応する炭水化物を生じ、所望ならば、損なわれないままの環系カルボン酸に至る。例は、α－１，４－グルカン－６－アルデヒド、β－１，４－グルカン－６－アルデヒド、β－２，１－フルクタン６－アルデヒド及びβ－２，６－フルクタン－１－アルデヒドを含む。これらの生成物は、アルデヒド基が例えば、アミン化合物などと更に反応する、官能基を含む炭水化物の有用な中間体である。これらは、また、架橋された炭水化物の有用な中間体でもあり、アルデヒド基が例えばジアミン試剤と反応する。

【００１１】

本発明により使用される触媒は、好適な酸化還元系の存在下で酸化可能である、酸化還元酵素または他の酵素である。本発明の方法で使用される酸化還元酵素、すなわち更なる酸化還元系の存在なしで酸化可能である酵素は、ペルオキシダーゼとオキシダーゼ、特にポリフェノールオキシダーゼとラッカーゼを含む。金属錯体、例えばバナジウム酸塩等の更なる酸化還元系が存在する場合には、フィターゼ及びリパーゼ等のしかるべきヒドロラーゼを使用することができる。例えば、リパーゼは、有機の、特にC_1－C_6カルボン酸（例えば、酢酸）の存在下でＴＥＭＰＯ／過酸化水素／銅により１級アルコール官能基を選択的酸化するのに有効な触媒であることが判明する。完全な酵素の代りに、いわゆる「合成酵素」、すなわち酵素を模倣する遷移金属錯体を使用することができる。このような錯体は、例えばバナジウム、マンガン、鉄、コバルト、ニッケルまたは銅を、錯化剤、特に２，２’－ビピリジル、フェナントロリン、テトラメチルエチレンジアミン、ペンタメチルジエチレントリアミン及び１，４，７－トリメチル－１，４，７－トリアゾナン、及びヒスチジンとそのオリゴマー等のこれらの環状相当品等のポリアミンと共に含んでなる。この金属活性化酵素は、最終の電子受容体として過酸化水素、アルキル及びアリール（アルキル）ハイドロパーオキサイド（ｔ－ブチルハイドロパーオキサイド等の）または塩素酸塩を必要とする。

【００１２】

本発明により使用することができるペルオキシダーゼ（ＥＣ１．１１．１．１－１．１１．１．１１）は、補助因子に無関係なペルオキシダーゼ、特に古典的なペルオキシダーゼ（ＥＣ１．１１．１．７）を含む。ペルオキシダーゼは、植物、バクテリア、線維状及び他の菌類及びイーストを含むいかなる起源からの由来であることができる。例は、ホースラデッシュペルオキシダーゼ、大豆皮ペルオキシダーゼ、骨髄ペルオキシダーゼ、ラクトペルオキシダーゼ、Ａｒｔｈｒｏｍｙｃｅｓ ａｎｄ Ｃｏｐｒｉｍｕｓペルオキシダーゼである。いくつかのペルオキシダーゼは市販されている。このペルオキシダーゼは、電子受容体として過酸化水素を必要とする。

【００１３】

ポリフェノールオキシダーゼ（ＥＣ１．１０．３．１）は、チロシナーゼ及びリグニンペルオキシダーゼ等のカテコールオキシダーゼを含む。好適なポリフェノールオキシダーゼは、菌類、植物または動物から得られてよい。ポリフェノールオキシダーゼは、電子受容体として酸素を必要とする。ラッカーゼ（ＥＣ１．１０．３．２）は、時にはポリフェノールオキシダーゼの下に置かれるが、これらは、また、時には明確な群として分類され得、ｐ－ジフェノールオキシダーゼと呼ばれる。ラッカーゼは、植物起源または微生物の、特に菌類の起源、例えばＴｒａｍｅｔｅｓ ｖｅｒｓｉｃｏｌｏｒの種由来であることができる。組み換えラッカーゼの使用が有利であることが可能である。また、このラッカーゼは、電子受容体として酸素を必要とする。

【００１４】

比較的温和な条件下で、例えば２と１０の間のｐＨ、また１５と６０℃の間の温度（双方とも個別の酵素または金属錯体に依って）で、本発明の方法を行うことができる。この反

応媒体は、水性媒体、または均一な混合媒体、例えばアルコール／水またはエーテル／水混合物、または不均一な媒体、例えば水と疎水性エーテル、炭水化物またはハロゲン化炭水化物等の水－非混和性有機溶媒との混合物であることができる。後者の場合には、酵素及び／またはニトロキシル及び酸化剤が水性相に存在し、アルコール基質とアルデヒドまたはケトン生成物が有機相に存在してよい。必要ならば、相間移動触媒を使用してよい。このタイプの反応は、例えば１９－ヒドロキシステロイドの選択的酸化等のステロイドの酸化及びアルデヒド及び／またはカルボキシル基の芳香化合物等の影響を受け易い他の化合物への導入に好適である。また、この反応媒体は、特にこのニトロキシルの酵素を固体キャリア上に固定化する場合には、固体／液体混合物であることもできる。基質または生成物が比較的影響を受け易い場合、あるいは生成物の他の試剤からの分離が困難である場合不均一な反応媒体は有利であることもある。

【００１５】

本発明は、また、本発明の方法により得られる新規な炭水化物酸化生成物及びこれらの誘導体にも関する。これらは、１００個、特に５０個、更には２５個のモノサッカライド単位当たり少なくとも１個のヒドロキシメチルがヘミアセタールまたは類似の形であってもなくとも、カルボアルデヒド基に変換された、ポリサッカライドを含む。但し、平均として、各分子がオリゴーあるいはポリサッカライドの還元性末端であり得る（ヘミアセタール化された）アルデヒド基以外の少なくとも１個のカルボアルデヒド基を含有するものとする。この炭水化物が澱粉である場合には、酸化度は、２５個のアンヒドログルコース単位当たり少なくとも１個のカルボアルデヒド基である。このカルボアルデヒド基は、好ましくは分枝単位によりも鎖（骨格）単位に存在する。１００（５０，２５）個単位当たりのこの少なくともカルボアルデヒド基には、ガラクトースオキシダーゼによる酸化により得ることができる、末端ガラクトース単位から誘導されるカルボアルデヒド基は含まれない。この新規な生成物は、グリコシド誘導体、すなわちアセタール化された末端基に加えて、非ーガラクトースヒドロキシメチレン基の酸化により得ることができる、少なくとも一つのカルボアルデヒド基を持つ生成物を含む。

【００１６】

本発明の製品においては、カルボアルデヒド基を含むモノサッカライド環は、大部分損なわれないままであり、アルデヒド基の数はカルボキシル基の数よりも多く、特に2倍以上多い（導入されたカルボキシアルキル基以外）。カルボキシル基迄の少なくとも部分的な更なる酸化を常に起こす従来技術の酸化方法によっては、このような製品は容易に製造されない。主含量のアルデヒド基を持つ唯一の普通の炭水化物誘導体は、澱粉、セルロースなどの過ヨウ素酸塩－タイプの酸化製品であり、ここではアルデヒド基を含む環が破壊されている。本発明により網羅されているアルデヒド炭水化物は、特に非セルロースタイプである。本発明により得ることができる製品は、アルデヒド基に加えて、他の官能基、更なる酸化により、あるいはカルボキシアルキル化（例えばクロロ酢酸との反応）により特にカルボキシル基を含んでよい。

【００１７】

本発明の新規な誘導体は、エマルションなどの濃化剤、増粘剤、安定剤として、そして特にアルコール、アミン、及びアルデヒド官能基との結合が可能である他の試剤と共に更なる官能基化のための出発材料として極めて好適である。このような試剤は、炭水化物を架橋するのに、あるいはこれらをアミノ酸、タンパク質、活性基などに結合するのに使用することができる、架橋剤（ジアミン、ジオールなど）を含む。

【００１８】

また、澱粉または木綿セルロース等のバイオポリマーを変成して、誘導体化（例えば、テキスタイルの染色、テキスタイル繊維の強化及びピリング防止）を可能にするのに、あるいは粘度及び他の物理的あるいは化学的性質を適合させるのに、例えばフルクタン、マンナン、セルロースを含む食物繊維を変成するのに、本発明の方法を使用するのも有利である。

【００１９】

本発明は、また、例えばアミンと上記に述べたアルデヒド炭水化物とを結合して、添付のクレームに定義されているような炭水化物のイミノあるいはアミノ誘導体を特に還元アミノ化により製造することによって得られる誘導体にも関する。また、更に誘導体化するために、このアルデヒド炭水化物をヒドロキシー官能基化された化合物、例えばグリコール酸により、反応し、アセタール化することができる。

実施例：全般

Ｂｌｕｍｅｎｋｒａｎｔｚらの方法（Ａｎａｌ．Ｂｉｏｃｈｅｍ．（１９７３）５４，４８４）を用い、濃硫酸中のホウ酸（０．０１２５Ｍ）を用い、３－ヒドロキシビフェニルを添加し、そして吸光度を５２０ｎｍで測定して、ウロン酸（ヘキソピラノース単位の６－ＣＯＯＨ）含量を求めた。

【００２０】

差し引き法（アルデヒドの塩素酸塩と過酸化水素による酸化の前後でウロン酸含量を定量）により、あるいはヒドロキシラミン塩酸塩を添加して、オキシムを生成し、そして遊離される塩酸を逆滴定することにより、あるいは^{13}Ｃ ＮＭＲスペクトル（アンヒドログルコース単位のＣ１に対するアルデヒドのＣ６シグナルの強度あるいはこのオキシム中のＣ６（Ｃ＝Ｎ）の強度）により、アルデヒド含量を求めた。

実施例１：ホースラデシュペルオキシダーゼを用いる６－アルデヒド澱粉の製造

２グラムの澱粉を１００ｍｌの水中１００℃でゼラチン化した。得られた溶液を２２℃迄冷却した。この溶液に２５ｍｇのＴＥＭＰＯ（０．１３ミリモル）と４０ｍｇのペルオキシダーゼ（ＨＲＰＯ）を添加した。ｐＨを酢酸（０．１Ｍ）により５に調節した。過酸化水素溶液（５０ｍｌ中の１．５ｍｌの３０％）を滴加した（時間当たり２ｍｌ）。ｐＨ調節は不必要であった。２５時間後、ヒドロキシルアンモニウムクロライドの添加により試料を分析した。この間接分析により、３０％のＣ６－アルデヒド澱粉が生成し、^{13}Ｃ ＮＭＲにより確認された。

実施例２：ラッカーゼによるプルランの酸化

１００ｍｌ水中の１．８４ｇのプルラン（１１．５ミリモルアンヒドログルコース単位）、１７ｍｇのＴｒａｍｅｔｅｓ ｖｅｒｓｉｃｏｌｏｒラッカーゼＶＩＩＩｂ（組み換え大腸菌中で発現，ＷａｃｋｅｒＣｈｅｍｉｅ）及び２５ｍｇのＴＥＭＰＯの溶液に、酸素ガスをバブリングした。この溶液（６．１）のｐＨは、２４時間後に４．５迄徐々に減少した。ヒドロキシルアミン塩酸塩との反応により求めたこの溶液のアルデヒド含量は、１．１ミリモルであった。このウロン酸含量は２４％であった。このアルデヒド基をカルボン酸基に酸化するために、この溶液を塩素酸ナトリウムと過酸化水素により処理した。処理後、ウロン酸は３２％迄増加した。酸化性基基準で、収率はそれぞれ３６と４８％であった。この溶液をエタノール中に注いだ。白色沈殿が生成し、１日後にこれを濾過により集め、真空中で乾燥した。この材料のウロン酸含量は２５％であった。

実施例３：ラッカーゼによるプルランの酸化

１．８４ｇのプルラン（１１．５ミリモル）、１００ｍｇの４－アセタミド－ＴＥＭＰＯ、及び１８ｍｇのラッカーゼ（Ｔ．ｖｅｒｓｉｃｏｌｏｒ）の溶液を作製した。この混合物を酢酸ナトリウム／酢酸緩衝液（０．０５Ｍ）により緩衝した。この溶液の初期のｐＨは６．１であった。この混合物を密閉系中で酸素ガスに曝露した。１日の反応後、２４ｍｌの酸素が消費された。ｐＨを元の値に戻すために、２ｍｌの０．５ＭＮａＯＨを添加した。この反応を更に１日続け、その結果２０ｍｌの酸素ガスが消費された。最終ｐＨは５．１であった。１．２ｍｌのＮａＯＨ（０．５Ｍ）の添加により、このｐＨを再度調節した。１５ｍｇラッカーゼを添加し、反応を２日間進行させた。この期間の後、ｐＨは４．５であり、３０ｍｌの酸素ガスが消費された。ｐＨを６とするために、３ｍｌの０．５ＮａＯＨを添加する必要があった。この溶液に０．２ｍｌの過酸化水素（３０％ｗ／ｗ）と２５０ｍｇの塩素酸ナトリウムを添加した。１日の反応後、ウロン酸含量を測定した。塩素酸ナトリウムによる酸化前のウロン酸の収率は、５５０ｍｇ（２６％）であり、後では６９５ｍｇ（３３％）であった。

実施例４：ラッカーゼを用いるＴＥＭＰＯのニトロソニウム塩の製造

ＴＥＭＰＯニトロソニウムイオンの溶液をラッカーゼにより次のように作製した。６．９ｇのＴＥＭＰＯを１ｌの脱塩水に溶解した。Ｔ．ｖｅｒｓｉｃｏｌｏｒ（Ｗａｃｋｅｒ）からの２００ｍｇのラッカーゼＶＩＩＩｂを２０ｍｌの脱塩水に懸濁した。この酵素溶液を１０分間撹拌した後、Ｐ６カラムを用いて遠心分離（５分　１５００ｘｇ）後の上澄み液を脱塩した。この脱塩した材料をＴＥＭＰＯ溶液に添加した。ほぼ１５０分間でｐＨ５のｐＨ一定の条件下、周囲温度で、空気噴霧のエアレーションにより、１００．８ｍｌのＨＣｌ（０．４Ｎ）の消費と黄色から更に橙色へのシフト（比Ｅ４８０／Ｅ４３０はほぼ０．３から１に増加する）により定量して、９１％のＴＥＭＰＯをニトロソニウムに変換した。

実施例５：ニトロソニウム塩とＵＦ膜システムを用いる澱粉の酸化

実施例４により得られるニトロソニウム溶液を０．２Ｍ酢酸塩によりｐＨ４．５で緩衝した。２ｇの土産のジャガイモ澱粉を１００ｍｌの水中でゼラチン化し、１００ｍｌの緩衝したニトロソニウム溶液と混合した。この混合物を２００ｍｌの撹拌したＵＦ容器中に注いだ（カットオフ５ｋＤ）。ほぼ８００ｍｌのニトロソニウム溶液を０．５ｍｌ／分の速度で室温２０℃で容器中にポンプ注入した。この透過液は、５０％のニトロソニウムイオンのＴＥＭＰＯへの変換（Ｅ４８０／Ｅ４３０比基準で）を示した。この処理の後、この澱粉のウロン酸含量は３８％であることが判明した。

実施例６：酸素／ラッカーゼ／ＴＥＭＰＯサイクルを用いる澱粉の変換

リントナーじゃがいも澱粉（Ｓｉｇｍａ５－２６３０）を水中にゼラチン化することにより、澱粉溶液を製造した。０．２Ｍコハク酸／コハク酸塩緩衝液の添加により、ｐＨを調節した。ＴＥＭＰＯまたは４－アセトアミドＴＥＭＰＯ（４ａｃｍＴ）を添加した。（ＴＥＭＰＯは、ある条件において、加工時に溶解する沈殿を澱粉と共に形成する）。大腸菌中で発現されたＴｒａｍｅｔｅｓ　ｖｅｒｓｉｃｏｌｏｒからのラッカーゼＶＩｌＩｂ（ＷａｃｋｅｒＣｈｅｍｉｅ製）を１０ｍｇ／ｍｌ０．２Ｍコハク酸塩緩衝液ｐＨ６で懸濁した。１０ｍｇのラッカーゼについて遠心分離（５分　１５００ｇ）した後、１ｍｌの上澄み液を添加した。撹拌した加圧容器ＡとＢ中でこの溶液への酸素移動を増進させた。双方の容器にはほぼ１００ｍｌが入っていた。ガス相との接触面積は、容器Ａについては７０ｃｍ^{2}であり、容器Ｂについては３２ｃｍ^{2}であった。Ｃ$_{6}$－酸化（アルデヒドまたはカルボン酸）に関する実験条件と結果を表１及び２に要約する。反応条件についての重要なパラメーターは、この溶液への酸素移動、ｐＨ、温度、ＴＥＭＰＯ、酵素及び澱粉の濃度である。ウロン酸の生成をＢｌｕｍｅｎｋｒａｎｔｚによりモニターした。次の条件下でウロン酸に酸化した後、アルデヒドの生成をモニターした。５ｍｌの試料（２０ｇ／ｌ澱粉）に０．０９５ｍｌの３％Ｈ$_{2}$Ｏ$_{2}$と０．５ｍｌの２０ｍｇ／ｍｌ塩素酸ナトリウムを添加した。このウロン酸含量を１６時間後室温で測定した。

【００２１】

【表１】

表1-1 容器A中で試験した影響の要約

空気	O2	澱粉ﾘﾝﾄﾅｰ	ﾗｯｶｰｾﾞ	pH	Tempo	4acmT[1]	T	時間	% COOH	% ald または hemi[2]
ﾊﾞｰﾙ	ﾊﾞｰﾙ	g/l	mg/100ml		g/l	g/l	°C	h		
2		10	100	6	1		25	45	73,4	nd
1,5		10	100	5		5	25	45	54,2	nd
	4	10	100	6	4		30	15	94,9	nd
2		10	100	6	4		30	15	100,0	nd
	1	10	100*	5,3		6	30	15	78,8	nd
2		10	10	6	4		30	20	60,3	4,9
	1	10	10*	6	4		30	20	50,0	5,1
	4	10	10	5,3		4	30	20	30,5	11,9
	4	20	10*	4,5		4	30	20	19,7	12,0
2		20	10	4		4	30	20	11,5	12,5

*20時間で酵素を容器中にﾎﾟﾝﾌﾟ注入

[1] 4acmT = 4-ｱｾﾄｱﾐﾄﾞ-TEMPO

[2] これらのｱﾙﾃﾞﾋﾄﾞまたはﾍﾐｱｾﾀｰﾙ

【００２２】
【表２】

表1-2 容器B中で試験した影響の要約

O2	澱粉ﾘﾝﾄﾅｰ	ﾗｯｶｰｾﾞ	pH	Tempo	4acmT	T	時間	% COOH	% ald または hemi
ﾊﾞｰﾙ	g/l	mg/100ml		g/l	g/l	°C	h		
4	10	100	6	4		30	15	94,9	nd
4	10	10	5,3		4	30	20	30,5	11,9
6	20	1	6		4	40	20	7,1	4,2

【００２３】
実施例７：ＴＥＭＰＯ／Ｍｎ／Ｈ$_2$Ｏ$_2$によるプルランの酸化

２５ｍｌの水中に２５０ｍｇのプルランと２０ｍｇのＴＥＭＰＯを溶解した。この溶液に２５ｍｇの硝酸マンガンを、続いて１００μｌの過酸化水素（３％溶液，ｗ／ｗ）とビピリジン溶液（５ｍｌ　０．０５Ｍ）を添加した。この反応をｐＨ６．５で行った。１日目に、６０ｍｇ（１．８ミリモル）の過酸化水素を添加し、１日後に２５ｍｇのウロン酸が生成した。２日目に、３０ｍｇの過酸化水素を添加し、ウロン酸の量は５０ｍｇに増加した。このアルデヒド基を過酸化水素／塩素酸ナトリウムによりカルボン酸基に変換し、この含量は９０ｍｇ（Ｄ．Ｏ．６０％）に上昇した。

【国際公開パンフレット】

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(54) Title: PROCESS FOR SELECTIVE OXIDATION OF PRIMARY ALCOHOLS AND NOVEL CARBOHYDRATE ALDEHYDES

(57) Abstract

A process for producing aldehydes, and/or carboxylic acids is described, in which a primary alcohol, especially a carbohydrate, is oxidised using a catalytic amount of a nitrosonium compound obtained by oxidising a nitroxyl compound in the presence of an enzyme compound capable of oxidation. Further described are oxidised carbohydrates containing at least 1 cyclic monosaccharide chain group carrying a carbaldehyde group per 25 monosaccharide units and per molecule.

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Process for selective oxidation of primary alcohols and novel carbohydrate aldehydes

[0001] The invention relates to the production of nitrosonium ions (oxoammonium ions) by oxidation of nitroxyl radicals, especially 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO). The nitrosonium ions can be used as a catalytic oxidising agent for the selective oxidation of primary alcohols to aldehydes.

[0002] Such a process in which TEMPO is reoxidised by chemical means is known from a review by De Nooy in *Synthesis* 1996, 1153-1174 and from WO 95/07303.

[0003] It was found according to the invention that oxidation of alcohol functions, especially primary alcohol functions, can be carried out without using chlorine-based oxidising agents and with the use of hydrogen peroxide or oxygen as the ultimate oxidising agent. The oxidation according to the invention is performed using enzymes and/or metal complexes. This oxidation, when carried out on primary alcohols, surprisingly results in formation of aldehydes, if desired without substantial further oxidation to carboxylic groups using appropriate conditions. The aldehydes may be present in the (hemi)acetal form and related structures. An adaptation of the oxidation process of the invention can be used to oxidise secondary alcohols, especially carbohydrates, to keto derivatives. The process of the invention is further defined by the characterising features of the appending claims.

[0004] The non-prepublished International patent applications WO 99/23117 and WO 99/23240 describe the oxidation of cellulose or starch, respectively, using an oxidative enzyme such as laccase with oxygen and TEMPO mediation. The laccase/TEMPO oxidation of cellulose resulted in the presence of a low and unspecified level carboxyl and carbonyl groups, while the laccase/TEMPO oxidation of starch was reported to yield a product having 1 carboxyl group and 3 aldehyde groups per 100 glucose units; no method of determining aldehyde content was given.

[0005] In the following description, reference is made to TEMPO only for the sake of simplicity, but it should be understood that other suitable nitroxyls, i.e. organic nitroxyl compounds lacking α-hydrogen atoms, such as 2,2,5,5-tetramethylpyrrolidine-N-oxyl (PROXYL), 4-hydroxy-TEMPO, 4-acetamido-TEMPO and derivatives thereof and those described in WO 95/07303 can be substituted for TEMPO. These di-tert-alkyl nitroxyls are especially suitable for selectively oxidising primary alcohols to aldehyde functions, in particular in the presence of secondary alcohol functions that should not be oxidised. Less sterically hindered nitroxyls, such as 4,4-dimethyloxazolidine-N-oxyl (DOXYL), are suitable for preferentially oxidising secondary alcohols to keto functions, for example in the production of keto cellulose or keto starch. The active oxidising species is the nitrosonium ion (oxoammonium ion $>N^{+}=O$), that is produced in situ by oxidation of the corresponding hydroxylamine and nitroxyl radical. If desired, the reaction can be performed in two steps, the production of the nitrosonium ion being the first and the oxidation of the alcohol function being the second.

[0006] A catalytic amount of nitroxyl is preferably 0.1-25% by weight, based on the primary alcohol, or 0.1-25 mol% with respect to the primary alcohol. The nitroxyl may also be immobilised, e.g. by coupling of the hydroxyl group of 4-hydroxy-TEMPO to a suitable carrier, or in the form of a polymeric nitroxyl such as:
$-[(CH_3)_2C\text{-}NO.\text{-}C(CH_3)_2\text{-}A\text{-}]_n\text{-}$, wherein A may be an alkylene group and/or a heteroatom, and n is a number form e.g. 10 up to several hundreds.

[0007] The process of the invention can be used for the oxidation of primary alcohols initially to the corresponding aldehydes. If required the primary products can be further oxidised to the corresponding carboxylic acids by using known oxidising agents such as hypochlorite, chlorite, hydrogen peroxide or by using TEMPO-mediated oxidation under more vigorous conditions such as an increased temperature e.g. from 40-80 °C, or for prolonged exposure to the reaction conditions. Alternatively, the aldehyde/carboxylic acid ratio can be increased by using relative low pH's (e.g. pH 3-7), by controlled addition of oxidising agent, by lowering the oxygen concentration, or by first preparing the nitrosonium ion solution (two-step process).

[0008] The present process is especially favourable for the selective oxidation of primary hydroxyl groups in alcohols having a secondary alcohol function in addition to the primary alcohol, such as 1,6-octanediol, 1,9-octadecanediol, steroid hormones, sugar alcohols, glycosides (flavour precursors), and in particular carbohydrates having primary alcohol functions. The carbohydrates may be monosaccharides, such as glucose, fructose, disaccharides, such as sucrose, maltose, lactose, oligosaccharides and polysaccharides. The oligo- and polysaccharides may be of any type, e.g. glucans such as starch, starch components (i.e. amylose, amylopectine, dextrins), pullulan (α-1,4-α-1,4-α-1,6-glucan), cellulose (in particular non-wood), chitin, lichenin etc., furanofructans such as inulin and levan, galactans, arabinogalactans, furanoid pentosans (xylans), (galacto)mannans (guar, locust bean gum), bacterial exopolysaccharides (EPS) and the like and derivatives of such carbohydrates, such as hydrolysates. These oligo- and polysaccharides include hetero-saccharides, i.e. those which have different structural units, even if those different units themselves may not have primary hydroxyl groups such as uronic acid units, e.g. in xanthan and carbohydrates derived form algae. The carbohydrates to be oxidised according to the invention include glycosides and other protected carbohydrates. Further examples are glyconic acids, such as lactobionic acid delta-lactone, that can be oxidised to glycaric acids and the like.

[0009] A distinct group of compounds suitable for oxidation with the present process consists of hydroxyalkylated carbohydrates such as hydroxypropyl cellulose, hydroxyethyl starch or hydroxyethylinulin, which result in an alternative way for producing formylalkyl carbohydrates. Other suitable carbohydrate substrates in which at least a part of the (6-) hydroxymethyl groups are intact, include for example (2- and 3-) carboxymethyl carbohydrates.

[0010] The oxidation of carbohydrates containing primary hydroxyl groups results in the corresponding carbohydrates containing aldehydes and, if desired, to carboxylic acids, with intact ring systems. Examples include α-1,4-glucan6-aldehydes, β-1,4-glucan-6-aldehydes, β-2,1-fructan6-aldehydes and β-2,6-fructan-1-aldehydes. These products are useful intermediates for functional carbohydrates wherein the aldehyde groups are further reacted with e.g. amine compounds and the like. They are also useful intermediates for crosslinked carbohydrates, in which the aldehyde groups are further reacted with e.g. diamine reagents.

[0011] The catalysts to be used according to the invention are oxidoreductases or other enzymes that are capable of oxidation in the presence of a suitable redox system. Oxidoreductases, i.e. enzymes capable of oxidation without the presence of further redox systems, to be used in the process of the invention include peroxidases and oxidases, in particular polyphenol oxidases and laccase. Certain hydrolases, such as phytase and lipases, can be used when a further redox system is present such as a metal complex, e.g. vanadate. For example, lipases are found to be effective catalysts for selective oxidation of primary alcohol functions with TEMPO / hydrogen peroxide / copper in the presence of an organic, in particular a C_1-C_6 carboxylic acid (e.g. acetic acid). Instead of complete enzymes, so-called "synzymes", i.e. transition metal complexes mimicking enzymes can be used. Such complexes comprise e.g. vanadium, manganese, iron, cobalt, nickel or copper with complexing agents, in particular polyamines, such as 2,2'-bipyridyl, phenanthroline, tetramethylethylenediamine, pentamethyldiethylenetriamine and their cyclic counterparts such as 1,4,7-trimethyl-1,4,7-triazonane, and histidine and its oligomers. The metal-assisted enzymes require hydrogen peroxide, alkyl and ar(alk)yl hydroperoxides (such as tert-butyl hydroperoxide) or chlorite as an ultimate electron acceptor.

[0012] Peroxidases (EC 1.11.1.1 - 1.11.1.11) that can be used according to the invention include the peroxidases which are cofactor-independent, in particular the classical peroxidases (EC 1.11.1.7). Peroxidases can be derived from any source, including plants, bacteria, filamentous and other fungi and yeasts. Examples are horseradish peroxidase, soy-hull peroxidase, myeloperoxidase, lactoperoxidase, *Arthromyces* and *Coprinus* peroxidases. Several peroxidases are commercially available. The peroxidases require hydrogen peroxide as an electron acceptor.

[0013] Polyphenol oxidases (EC 1.10.3.1) include tyrosinases and catechol oxidases, such as lignine peroxidase. Suitable polyphenol oxidases may be obtained from fungi, plants or animals. The polyphenol oxidases require oxygen as an electron acceptor. Laccases (EC 1.10.3.2) are sometimes grouped under the polyphenol oxidases, but they can also be classified as a distinct group, sometimes referred to as p-diphenol oxidases. Laccases can be derived from plant sources or from microbial, especially fungal, sources, e.g. of the species *Trametes versicolor*. The use of recombinant laccases can be advantageous. The laccases also require oxygen as an electron acceptor.

[0014] The process of the invention can be performed under relatively mild conditions, e.g. at a pH between 2 and 10, and at a temperature between 15 and 60°C (both depending on the particular enzyme or metal complex). The reaction medium can be an aqueous medium, or a homogeneous mixed medium, e.g. of an alcohol/water or an ether/water mixture, or a heterogeneous medium, e.g. a mixture of water and a water-immiscible organic solvent such as a hydrophobic ether, a hydrocarbon or a halogenated hydrocarbon. In the latter case, the enzyme and/or the nitroxyl and the oxidising agent may be present in the aqueous phase and the alcohol substrate and the aldehyde or ketone product may be present in the organic phase. If necessary, a phase transfer catalyst may be used. This type of reaction is suitable e.g. for the oxidation of steroids, such as the selective oxidation of 19-hydroxy steroids, and the introduction of aldehyde and/or carboxylic groups into other sensitive compounds such as flavour compounds. The reaction medium can also be a solid/liquid mixture, in particular when the enzyme of the nitroxyl are immobilised on a solid carrier. A heterogeneous reaction medium may be advantageous when the substrate or the product is relatively sensitive or when separation of the product from the other reagents may present difficulties.

[0015] The invention also pertains to novel carbohydrate oxidation products and derivatives thereof obtainable with the process of the invention. These include polysaccharides in which at least 1 hydroxymethyl per 100, especially per 50 or even per 25, monosaccharide units has been converted to a carbaldehyde group, whether or not in hemiacetal or similar form, with the proviso that on average each molecule contains at least 1 carbaldehyde group other than a possible (hemiacetalised) aldehyde group at the reducing end of an oligo- or polysaccharide. When the carbohydrate is starch, the degree of oxidation is at least one carbaldehyde group per 25 anhydroglucose units. The carbaldehyde group is preferably present in chain (backbone) units, rather than in branch units. Not included in this at least carbaldehyde group per 100 (50, 25) units are carbaldehyde groups derived from terminal galactose units, which are obtainable by oxidation with galactose oxidase. The novel products include glycoside derivatives, i.e. products which, in addition to an acetalised end group have at least one carbaldehyde group obtainable by oxidation of non-galactose hydroxymethylene groups.

[0016] In the products of the invention, the monosaccharide rings that carry the carbaldehyde group are largely intact, and the number of aldehyde groups is greater, especially more than two times greater, than the number of carboxyl groups (other than introduced carboxyalkyl groups). Such products are not easily produce by prior art oxidation methods, which invariably lead to at least partial further oxidation to carboxyl groups. The only common carbohydrate derivatives having a predominant content of aldehyde groups are periodate-type oxidation products of starch, cellulose and the like, in which the rings bearing the aldehyde groups are broken. The aldehyde carbohydrates covered by the present invention are in particular of the non-cellulose type. The products obtainable according to the invention may contain, in addition to the aldehyde groups, other functional groups, especially carboxyl groups obtained by further oxidation or by carboxyalkylation (e.g. reaction with chloroacetic acid).

[0017] The novel derivatives of the invention are very suitable as thickeners, viscosifiers, stabilisers for emulsions and the like, and especially as starting materials for further functionalisation, especially with alcohols, amines, and other agents capable of coupling with an aldehyde function. Such agents include crosslinking agents (diamines, diols and the like), which can be used to crosslink the carbohydrates or to couple them to amino acids, proteins, active groups etc.

[0018] The process of the invention can also advantageously be used for modifying biopolymers such as starch or cotton cellulose, to allow derivatisation (e.g. dyeing of textile, strengthening of textile fibres and anti-pilling) or to adapt viscosity and other physical or chemical properties, for example to modify dietary fibres including fructans, mannans, cellulose etc.

[0019] The invention also pertains to derivatives obtained by coupling of the aldehyde carbohydrates described above with e.g. amines, especially by reductive amination, to produce imino or amino derivatives of carbohydrates as defined in the appending claims. Also, the aldehyde carbohydrates can be reacted acetalised with hydroxy-functionalised compounds, e.g. glycolic acid, for further derivatisation.

Examples: *General*

[0020] Uronic acid (6-COOH of hexopyranose units) contents were determined using the Blumenkrantz et al. method (*Anal. Biochem.* (1973) **54**, 484), using boric acid (0.0125 M) in concentrated sulphuric acid, adding 3-hydroxybiphenyl and measuring the extinction is measured at 520 nm.

[0021] Aldehyde contents were determined either by a subtractive method (determining the uronic acid content before and after of oxidation of aldehydes with chlorite and hydrogen peroxide), or by addition of hydroxylamine hydrochloride to produce an oxime and back-titration of liberated hydrochloric acid, or by ^{13}C NMR spectroscopy (intensity of C6 signal of aldehyde with respect to C1 of anhydroglucose unit, or intensity of C6 (C=N) in the oxime).

Example 1: *Production of 6-aldehyde starch using horse radish peroxidase*

[0022] Two grams of starch were gelatinised in 100 ml of water at 100°C. The solution obtained was cooled to 22°C. To this solution were added 25 mg TEMPO (0.13 mmol) and 40 mg of peroxidase (HRPO). The pH was adjusted to 5 with acetic acid (0.1 M). A hydrogen peroxide solution (1.5 ml 30% in 50 ml) was added drop-wise (2 ml per h). No pH adjustment was necessary. After 25 h a sample was analysed by addition of hydroxylammonium chloride. According to this indirect analysis, 30% of C6-aldehyde starch was formed, which was confirmed by ^{13}C NMR.

Example 2: *Oxidation of pullulan with laccase*

[0023] Through a solution of 1.84 g of pullulan (11.5 mmol anhydroglucose units) 17 mg of *Trametes versicolor* laccase VIIIb (expressed in recombinant *E. coli*, Wacker Chemie) and 25 mg of TEMPO in 100 ml water, oxygen gas was bubbled. The pH of the solution (6.1) decreased gradually to 4.5 after 24 hours. The aldehyde content of the solution determined by reaction with hydroxylamine hydrochloride was 1.1 mmol. The uronic acid content was 24%. To oxidise the aldehyde groups to carboxylic acid groups, the solution was treated with sodium chlorite and hydrogen peroxide. After treatment the uronic acid was increased to 32%. Based on the oxidisable groups the yields are 36 and 48 %, respectively. The solution was poured out into ethanol. A white precipitate was formed, which after one day was collected by filtration and dried in vacuum. The uronic acid content of this material was 25%.

Example 3: *Oxidation of pullulan with laccase*

[0024] A solution of 1.84 g pullulan (11.5 mmol), 100 mg 4-acetamido-TEMPO, and 18 mg laccase (*T. versicolor*) was prepared. The mixture was buffered with sodium acetate / acetic acid buffer (0.05M). The initial pH of the solution was 6.1. This mixture was exposed to oxygen gas in a closed system. After one day reaction 24 ml of oxygen was consumed. To bring the pH to its original value 2 ml 0.5 M NaOH was added. The reaction was continued for another day, resulting in the consumption of 20 ml of oxygen gas. The final pH was 5.1. The pH was adjusted again by addition of 1.2 ml NaOH (0.5 M). 15 mg laccase was added and the reaction was allowed to proceed for two days. After this period the pH was 4.5 and 30 ml oxygen gas was consumed. To bring the pH to 6, 3 ml 0.5 NaOH had to be added. To the solution 0.2 ml hydrogen peroxide (30% w/w) and 250 mg sodium chlorite were added. After one day reaction the uronic acid content was measured. The yield of uronic acid before oxidation with sodium chlorite was 550 mg (26%) and after 695 mg (33%).

Example 4: *Preparation of the nitrosonium salt of TEMPO using laccase*

[0025] A solution of TEMPO nitrosonium ion was made with laccase as follow. 6.9 g TEMPO was dissolved in 1 l demi water. 200 mg laccase VIIIb from *T. versicolor* (Wacker) was suspended in 20 ml demi water. After stirring the enzyme solution for 10 minutes, the supernatant after centrifugation (5 min 1500xg) was desalted using a P6 column. The desalted material was added to the TEMPO solution. In approximately 150 minutes under pH stat conditions at pH 5, ambient temperature, aerated with air sparge, 91 % of the TEMPO was converted to nitrosonium, as determined by the consumption of 100.8 ml of HCl (0,4 N) and a shift from a yellow to a more orange colour (the ratio E480/ E430 increases from approximately 0.3 to 1).

Example 5: *Oxidation of starch using nitrosonium salt and a UF membrane system.*

[0026] The nitrosonium solution obtained according to example 4 was buffered with 0.2 M acetate at pH 4.5. 2 g native potato starch was gelatinised in 100 ml water and mixed with 100 ml of the buffered nitrosonium solution. The mixture was poured into a 200 ml stirred UF vessel (cut-off 5 kD). Approximately 800 ml of the nitrosonium solution was pumped into the vessel at a rate of 0.5 ml/min. at room temp 20 °C. The permeate indicated a conversion of 50% of the nitrosonium ion bakc to TEMPO (based on the E480/E430 ratio). After this treatment, the uronic acid content of the starch was found to be 38%.

Example 6: *Conversion of starch using oxygen / laccase / TEMPO cycle*

[0027] Starch solutions were prepared by gelatinising Lintner potato starch (Sigma S-2630) in water. The pH was adjusted by addition of 0.2 M succinic acid / succinate buffer. Tempo or 4-acetamido tempo (4acmT) was added. (TEMPO forms a precipitate with starch in some conditions, which dissolves during the process.) Laccase VIIIb from *Trametes versicolor* expressed in *E.coli* (from Wacker Chemie) was suspended at 10 mg/ml 0,2 M succinate buffer pH 6. After centrifugation (5 min 1500g) for 10 mg laccase 1 ml of the supernatant was added. The oxygen transfer to the solution was enhanced in stirred pressurised vessels A en B. Both vessels contained approximately 100 ml. The area of contact with the gas phase was 70 cm^2 for vessel A and 32 cm^2 for vessel B. The experimental conditions and the results with regard to C_6-oxidation (aldehyde or carboxylic acid) are summarised in tables 1 and 2. Important parameters for the reaction conditions are:

- oxygen transfer to the solution, pH, temperature, concentration of TEMPO, enzyme and starch .

The formation of uronic acids was monitored according to Blumenkrantz. The formation of aldehydes was monitored after oxidation to uronic acids under the following conditions:

To 5 ml sample (20 g/l starch) 0.095 ml 3% H_2O_2 and 0.5 ml 20 mg/ml sodium chlorite was added. The uronic acid content was measured after 16 h at room temperature.

table 1-1. Summary of influences studied in vessel A

air bar	O2 bar	Starch lintner g/l	Laccase mg/100ml	pH	Tempo g/l	4acmT[1] g/l	T °C	Time h	% COOH	% ald or hemi[2]
2		10	100	6	1		25	45	73,4	nd
1,5		10	100	5		5	25	45	54,2	nd
	4	10	100	6	4		30	15	94,9	nd
2		10	100	6	4		30	15	100,0	nd
	1	10	100*	5,3		6	30	15	78,8	nd
2		10	10	6	4		30	20	60,3	4,9
	1	10	10*	6	4		30	20	50,0	5,1
	4	10	10	5,3		4	30	20	30,5	11,9
	4	20	10*	4,5		4	30	20	19,7	12,0
2		20	10	4		4	30	20	11,5	12,5

* the enzyme was pumped into the vessel during 20 h

[1] 4acmT = 4-acetamido-TEMPO

[2] aldehyde or hemiacetal thereof

table 1-2 Summary of influences studied in vessel B

O2	Starch lintner	laccase	pH	Tempo	4acmT	T	time	% COOH	% ald or hemi
bar	g/l	mg/100ml		g/l	g/l	°C	h		
4	10	100	6	4		30	15	94,9	nd
4	10	10	5,3		4	30	20	30,5	11,9
6	20	1	6		4	40	20	7,1	4,2

Example 7: *Oxidation of pullulan by TEMPO / Mn /* H_2O_2

In 25 ml of water 250 mg pullulan and 20 mg of TEMPO were dissolved. To this solution 25 mg manganese nitrate was added, followed by 100 µl of hydrogen peroxide (3% solution, w/w) and bipyridine solution (5 ml 0.05 M). The reaction was conducted at pH 6.5. At the first day 60 mg (1.8 mmol) hydrogen peroxide was added and after one day 25 mg of uronic acid was formed. During the second day 30 mg hydrogen peroxide was added and the amount of uronic acid was increased to 50 mg. The aldehyde groups were converted into carboxylic acid groups with hydrogen peroxide/sodium chlorite the content raised to 90 mg. (D.O. 60%).

Claims

1. A process for producing nitrosonium ions by oxidising a nitroxyl compound with an oxidising agent, *characterised* in that the nitroxyl compound is oxidised in the presence of an enzyme capable of oxidation and/or in the presence of a metal complex.

2. A process according to Claim 1, wherein the nitroxyl compound is a di-tert-nitroxyl compound, especially 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO).

3. A process according to Claim 1 or 2, wherein the enzyme capable of oxidation is an oxidoreductase.

4. A process according to Claim 3, wherein the enzyme is a peroxidase, especially horse radish, soy-bean, lignin peroxidase or myelo- or lacto-peroxidase, and the oxidising agent is hydrogen peroxide.

5. A process according to Claim 3, wherein the enzyme is a polyphenol oxidase or a laccase and the oxidising agent is oxygen.

6. A process according to Claim 1 or 2, wherein the enzyme is a hydrolase, especially phytase or lipase, in the presence of a metal compound.

7. A process for oxidising a primary alcohol with a nitrosonium ion as a catalyst, *characterised* in that the nitrosonium ion is produced by the process according to any one of Claims 1-6.

8. A process according to Claim 7, wherein the primary alcohol is comprised in a carbohydrate, especially an α-glucan or fructan or a derivative thereof.

9. A process according to Claim 8, wherein a carbonyl-containing carbohydrate containing at least 1 cyclic monosaccharide chain group carrying a carbaldehyde group per 25 monosaccharide units and per average molecule is produced.

10. A process according to Claim 8 or 9, wherein the carbohydrate is a hydroxyalkylated carbohydrate or a glycoside or a glyconic acid.

11. A process according to any one of Claims 1-7, wherein the primary alcohol is comprised in a steroid compound.

12. A process for treating textile fibres to introduce aldehyde groups, *characterised* in that the cotton fibres are treated with nitrosonium ion produced by the process according to any one of Claims 1-6.

13. An oxidised carbohydrate, the carbohydrate being selected from disaccharides, oligosaccharides and polysaccharides of the glucan, mannan, galactan, fructan, and chitin types and carbohydrate glycosides, containing at least 1 cyclic monosaccharide chain group carrying a carbaldehyde group per 25 monosaccharide units and per average molecule, or a chemical derivative thereof.

14. An oxidised carbohydrate according to Claim 13, containing at least 5 monosaccharide units per average molecule.

15. An oxidised carbohydrate according to Claim 13 or 14, which contains 1 to 50 cyclic monosaccharide chain group carrying a carbaldehyde group per 50 monosaccharide units and per average molecule.

16. A carbohydrate derivative according to any one of Claims 13-15, in which derivative at least a part of the carbaldehyde groups has been converted to a group with the formula $-CH=N-R$ or $-CH_2-NHR$, wherein R is hydrogen, hydroxyl, amino, or a group R^1, OR^1 or NHR^1, in which R^1 is C_1-C_{20} alkyl, C_1-C_{20} acyl, a carbohydrate residue, or group coupled with or capable of coupling with a carbohydrate residue.

17. A carbohydrate derivative according to any one of Claims 13-15, in which derivative at least a part of the carbaldehyde groups has been converted to a group with the formula $-CH(OR^3)-O-CH_2-COOR^2$ or $-CH(-O-CH_2-COOR^2)_2$, in which R^2 is hydrogen, a metal cation or an optionally substituted ammonium group, and R^3 is hydrogen or a direct bond to the oxygen atom of a dehydrogenated hydroxyl group of the carbohydrate.

18. A carbohydrate according to any one of Claims 13-17, further containing carboxyl and/or carboxymethyl groups.

【国際公開パンフレット（コレクトバージョン）】

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) **World Intellectual Property Organization**
International Bureau

(43) **International Publication Date**
31 August 2000 (31.08.2000)

PCT

(10) **International Publication Number**
WO 00/50621 A3

(51) **International Patent Classification**^{7}: **C12P 1/00**, 19/00, 13/00, 7/24, C07C 45/29, 45/32, 45/38, 45/39, C07H 1/00, C08B 1/00, C07H 3/00, 5/04, C08L 1/00, 3/00

(21) **International Application Number:** PCT/NL00/00117

(22) **International Filing Date:** 24 February 2000 (24.02.2000)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
99200536.3 24 February 1999 (24.02.1999) EP

(71) **Applicant** *(for all designated States except US)*: **NEDERLANDSE ORGANISATIE VOOR TOEGEPAST-NATUURWETENSCHAPPELIJK ONDERZOEK TNO** [NL/NL]; Schoemakersstraat 97, NL-2628 VK Delft (NL).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **JETTEN, Jan, Matthijs** [NL/NL]; Costerlaan 3 B, NL-3701 JL Zeist (NL). **VAN DEN DOOL, Ronald, Tako, Marinus** [NL/NL]; Dalkruid 1, NL-4102 KR Culemborg (NL). **VAN HARTINGSVELDT, Wim** [NL/NL]; Victor Hortastraat 27, NL-3822 VM Amersfoort (NL). **VAN WANDELEN, Mario, Tarcisius, Ragmandus** [NL/NL]; Rooseveltlaan 14, NL-3705 PG Zeist (NL).

(74) **Agent: JORRITSMA, Ruurd**; Nederlandsch Octrooibureau, Scheveningseweg 82, P.O. Box 29720, NL-2502 LS The Hague (NL).

(81) **Designated States** *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) **Date of publication of the international search report:**
8 November 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/50621 A3

(54) **Title:** PROCESS FOR PRODUCING NITROSONIOM IONS FROM A NITROXYL COMPOUND, THEIR APPLICATION IN THE SELECTIVE OXIDATION OF PRIMARY ALCOHOLS, AND NOVEL CARBOHYDRATE ALDEHYDES

(57) **Abstract:** A process for producing aldehydes, and/or carboxylic acids is described, in which a primary alcohol, especially a carbohydrate, is oxidised using a catalytic amount of a nitrosonium compound obtained by oxidising a nitroxyl compound in the presence of an enzyme compound capable of oxidation. Further described are oxidised carbohydrates containing at least 1 cyclic monosaccharide chain group carrying a carbaldehyde group per 25 monosaccharide units and per molecule.

【国際公開パンフレット（コレクトバージョン）】

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
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PCT

(10) International Publication Number
WO 00/50621 A3

(51) **International Patent Classification**^{7}: C12P 1/00, 19/00, 13/00, 7/24, C07C 45/29, 45/32, 45/38, 45/39, C07H 1/00, C08B 1/00, C07H 3/00, 5/04, C08L 1/00, 3/00

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(71) **Applicant** *(for all designated States except US)*: **NEDERLANDSE ORGANISATIE VOOR TOEGEPAST-NATUURWETENSCHAPPELIJK ONDERZOEK TNO** [NL/NL]; Schoemakersstraat 97, NL-2628 VK Delft (NL).

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(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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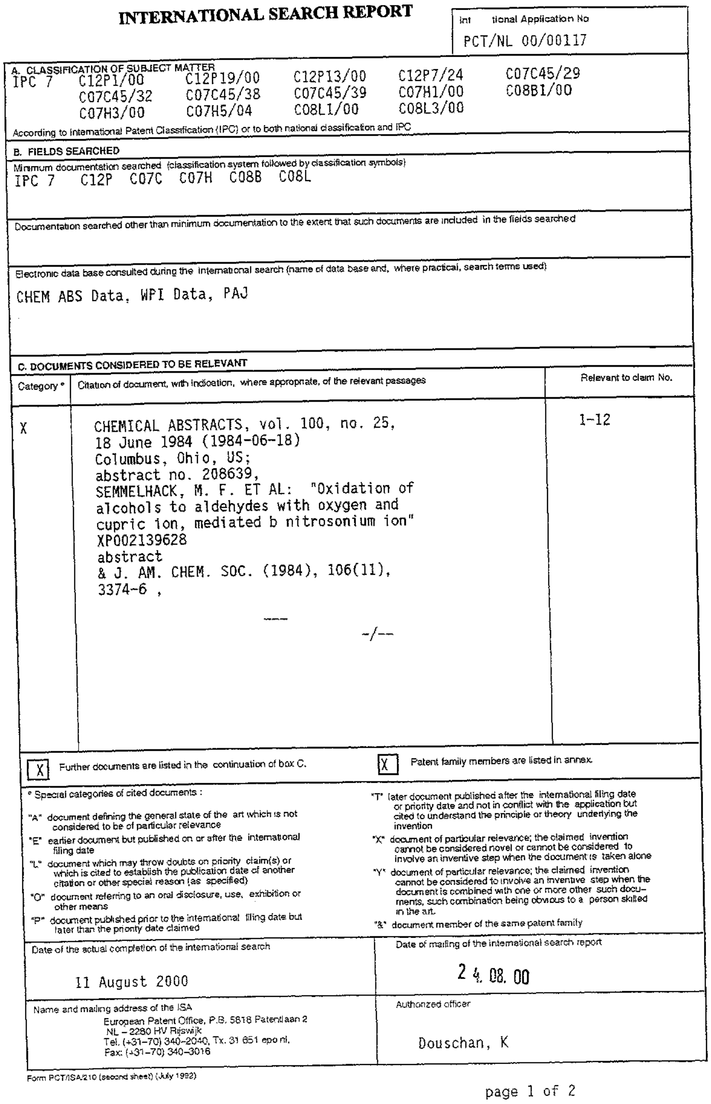
(57) **Abstract:** A process for producing aldehydes, and/or carboxylic acids is described, in which a primary alcohol, especially a carbohydrate, is oxidised using a catalytic amount of a nitrosonium compound obtained by oxidising a nitroxyl compound in the presence of an enzyme compound capable of oxidation. Further described are oxidised carbohydrates containing at least 1 cyclic monosaccharide chain group carrying a carbaldehyde group per 25 monosaccharide units and per molecule.

【国際調査報告】



INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/NL 00/00117

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12P1/00 C12P19/00 C12P13/00 C12P7/24 C07C45/29
C07C45/32 C07C45/38 C07C45/39 C07H1/00 C08B1/00
C07H3/00 C07H5/04 C08L1/00 C08L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12P C07C C07H C08B C08L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 100, no. 25, 18 June 1984 (1984-06-18) Columbus, Ohio, US; abstract no. 208639, SEMMELHACK, M. F. ET AL: "Oxidation of alcohols to aldehydes with oxygen and cupric ion, mediated b nitrosonium ion" XP002139628 abstract & J. AM. CHEM. SOC. (1984), 106(11), 3374-6 , --- -/--	1-12

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search 11 August 2000	Date of mailing of the international search report 24.08.00
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Douschan, K

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page 1 of 2

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/NL 00/00117

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BOBBITT J M ET AL: "ORGANIC NITROSINIUM SALTS AS OXIDANTS IN ORGANIC CHEMISTRY" HETEROCYCLES,XX,XX, vol. 27, no. 2, 1 January 1988 (1988-01-01), pages 509-533, XP000609709 ISSN: 0385-5414 see especially Table 1 the whole document	1-12
X	NOOY DE A E J ET AL: "HIGHLY SELECTIVE TEMPO MEDIATED OXIDATION OF PRIMARY ALCOHOL GROUPSIN POLYSACCHARIDES" RECUEIL DES TRAVAUX CHIMIQUES DES PAYS-BAS,NL,ELSEVIER SCIENCE PUBLISHERS. AMSTERDAM, vol. 113, no. 3, 1 March 1994 (1994-03-01), pages 165-166, XP000560836 ISSN: 0165-0513 the whole document	1-18
X	NOOY DE A E J ET AL: "ON THE USE OF STABLE ORGANIC NITROXYL RADICALS FOR THE OXIDATION OFPRIMARY AND SECONDARY ALCOHOLS" SYNTHESIS,DE,GEORG THIEME VERLAG. STUTTGART, 1 October 1996 (1996-10-01), pages 1153-1174, XP002072173 ISSN: 0039-7881 the whole document	1-12
X,P	WO 99 23117 A (VALTON TEKNILLINEN TUTKIMUSKESKUS) 14 May 1999 (1999-05-14) the whole document	1-12
X	US 3 632 802 A (J. N. BEMILLER ET AL.) 4 January 1972 (1972-01-04) claims 1,8; example 5	13-18
X	US 5 747 658 A (INSTITUT VOOR AGROTECHNOLOGISCH ONDERZOEK) 5 May 1998 (1998-05-05) claims 10,11; example 1	13-18
A	EP 0 124 439 A (INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE) 7 November 1984 (1984-11-07) the whole document	13-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 00/00117

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

International Application No. PCT/NL 00 /00117

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-12

   Claims 1-6 concern a process for producing nitrosonium ions by oxidising a nitroxyl compound in the presence of an enzyme and/or a metal complex.
   Claims 7-11 claim the use of the nitrosonium ions prepared in a process according to claims 1-6 for oxidising a primary alcohol, which is not specific and can be either from a carbohydrate or a steroid, etc.
   Claim 12 describes the application of the process of claims 7-11 in a process for treating textile fibres.

2. Claims: 13-18

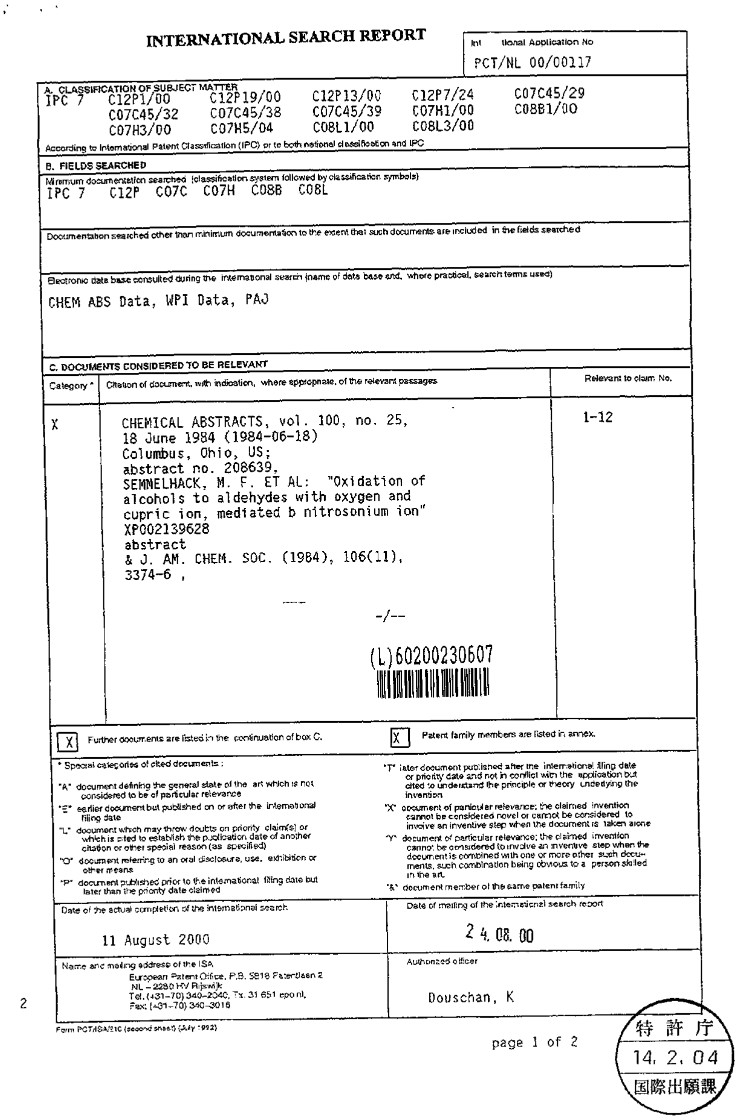
   Claims 13-18 concern an oxidised carbohydrate having specific characteristics as defined in claim 13 and containing at least one "cyclic monosaccharide chain group carrying a carbaldehyde group". The said oxidised carbohydrate "can" be prepared by the process of claims 7-11 (cf. p. 4 of the description), but is not limited thereto. The oxidised carbohydrates may also be further processed or derivatised (see claims 16-18). The products claimed in claims 13-18 are useful as thickeners, viscosifiers, stabilisers for emulsions and starting materials for further functionalisation.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter onal Application No
PCT/NL 00/00117

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 9923117	A	14-05-1999	FI	974139	A	05-05-1999
			AU	1035199	A	24-05-1999
US 3632802	A	04-01-1972	NONE			
US 5747658	A	05-05-1998	NL	9301905	A	01-06-1995
			AU	1123595	A	23-05-1995
			CA	2175794	A	11-05-1995
			EP	0726916	A	21-08-1996
			FI	961905	A	03-07-1996
			JP	9500414	T	14-01-1997
			WO	9512619	A	11-05-1995
EP 0124439	A	07-11-1984	FR	2545101	A	02-11-1984
			DE	3461938	D	12-02-1987
			JP	59205949	A	21-11-1984
			US	4672034	A	09-06-1987



INTERNATIONAL SEARCH REPORT

Int　tional Application No
PCT/NL 00/00117

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12P1/00 C12P19/00 C12P13/00 C12P7/24 C07C45/29
C07C45/32 C07C45/38 C07C45/39 C07H1/00 C08B1/00
C07H3/00 C07H5/04 C08L1/00 C08L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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(L)60200230607

☒ Further documents are listed in the continuation of box C.　　☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search 11 August 2000	Date of mailing of the international search report 24.08.00
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Douschan, K

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Form PCT/ISA/210 (second sheet) (July 1992)

page 1 of 2

2

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/NL 00/00117

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BOBBITT J M ET AL: "ORGANIC NITROSINIUM SALTS AS OXIDANTS IN ORGANIC CHEMISTRY" HETEROCYCLES,XX,XX, vol. 27, no. 2, 1 January 1988 (1988-01-01), pages 509-533, XP000609709 ISSN: 0385-5414 see especially Table 1 the whole document ---	1-12
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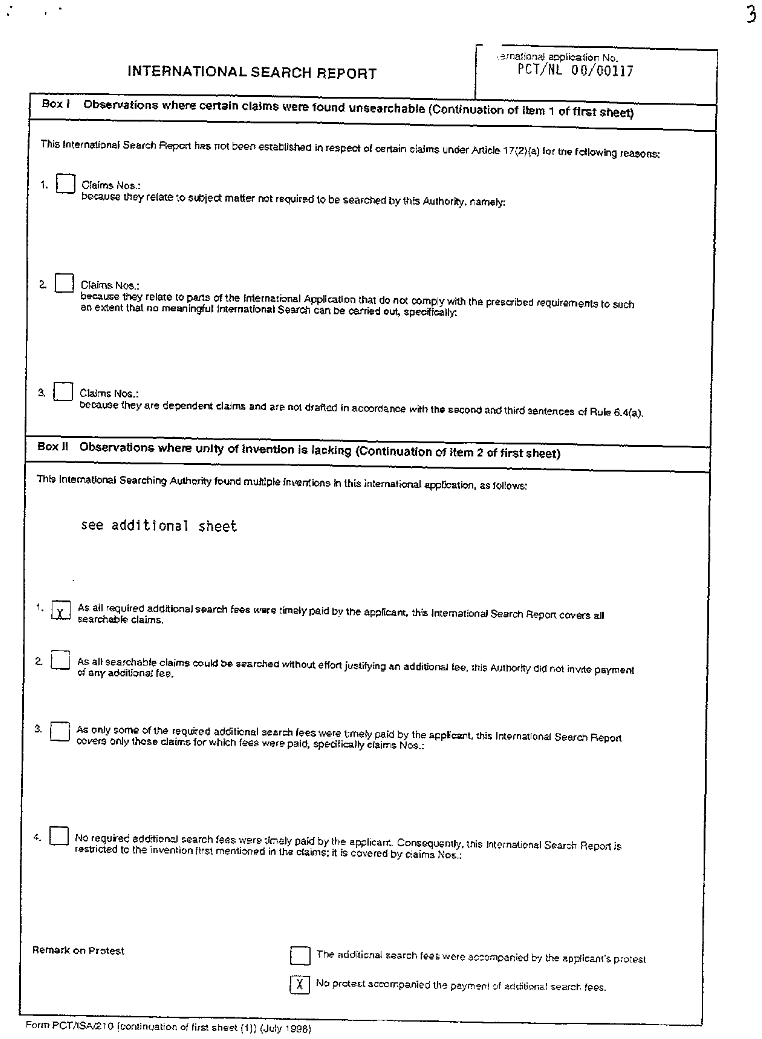

3

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/NL 00/00117

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

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Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest

☒ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

4

International Application No. PCT/NL 00 /00117

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-12

Claims 1-6 concern a process for producing nitrosonium ions by oxidising a nitroxyl compound in the presence of an enzyme and/or a metal complex.
Claims 7-11 claim the use of the nitrosonium ions prepared in a process according to claims 1-6 for oxidising a primary alcohol, which is not specific and can be either from a carbohydrate or a steroid, etc.
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5

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/NL 00/00117

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9923117	A	14-05-1999	FI	974139 A	05-05-1999
			AU	1035199 A	24-05-1999
US 3632802	A	04-01-1972	NONE		
US 5747658	A	05-05-1998	NL	9301905 A	01-06-1995
			AU	1123595 A	23-05-1995
			CA	2175794 A	11-05-1995
			EP	0726916 A	21-08-1996
			FI	961905 A	03-07-1996
			JP	9500414 T	14-01-1997
			WO	9512619 A	11-05-1995
EP 0124439	A	07-11-1984	FR	2545101 A	02-11-1984
			DE	3461938 D	12-02-1987
			JP	59205949 A	21-11-1984
			US	4672034 A	09-06-1987

Form PCT/ISA/210 (patent family annex) (July 1992)

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PATENT COOPERATION TREATY

PCT

REC'D 15 MAY 2001
WIPO PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference BO 42503	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/NL 00/00117	International filing date *(day/month/year)* 24/02/2000	(Earliest) Priority Date *(day/month/year)* 24/02/1999
Applicant NEDERLANDSE ORGANISATIE VOOR TOEGEPAST-... et al		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   - a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
     - ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   - b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :
     - ☐ contained in the international application in written form.
     - ☐ filed together with the international application in computer readable form.
     - ☐ furnished subsequently to this Authority in written form.
     - ☐ furnished subsequently to this Authority in computer readble form.
     - ☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
     - ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished
2. ☐ **Certain claims were found unsearchable** (See Box I).
3. ☒ **Unity of invention is lacking** (see Box II).
4. With regard to the **title**,
   - ☐ the text is approved as submitted by the applicant.
   - ☒ the text has been established by this Authority to read as follows:

PROCESS FOR PRODUCING NITROSONIOM IONS FROM A NITROXYL COMPOUND, THEIR APPLICATION IN THE SELECTIVE OXIDATION OF PRIMARY ALCOHOLS, AND NOVEL CARBOHYDRATE ALDEHYDES

5. With regard to the **abstract**,
   - ☒ the text is approved as submitted by the applicant.
   - ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. The figure of the **drawings** to be published with the abstract is Figure No. ______
   - ☐ as suggested by the applicant.
   - ☐ because the applicant failed to suggest a figure.
   - ☐ because this figure better characterizes the invention.
   - ☒ None of the figures.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 00/00117

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest

☒ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-12

Claims 1-6 concern a process for producing nitrosonium ions by oxidising a nitroxyl compound in the presence of an enzyme and/or a metal complex.
Claims 7-11 claim the use of the nitrosonium ions prepared in a process according to claims 1-6 for oxidising a primary alcohol, which is not specific and can be either from a carbohydrate or a steroid, etc.
Claim 12 describes the application of the process of claims 7-11 in a process for treating textile fibres.

2. Claims: 13-18

Claims 13-18 concern an oxidised carbohydrate having specific characteristics as defined in claim 13 and containing at least one "cyclic monosaccharide chain group carrying a carbaldehyde group". The said oxidised carbohydrate "can" be prepared by the process of claims 7-11 (cf. p. 4 of the description), but is not limited thereto. The oxidised carbohydrates may also be further processed or derivatised (see claims 16-18). The products claimed in claims 13-18 are useful as thickeners, viscosifiers, stabilisers for emulsions and starting materials for further functionalisation.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 00/00117

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12P1/00 C12P19/00 C12P13/00 C12P7/24 C07C45/29
C07C45/32 C07C45/38 C07C45/39 C07H1/00 C08B1/00
C07H3/00 C07H5/04 C08L1/00 C08L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12P C07C C07H C08B C08L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 100, no. 25, 18 June 1984 (1984-06-18) Columbus, Ohio, US; abstract no. 208639, SEMMELHACK, M. F. ET AL: "Oxidation of alcohols to aldehydes with oxygen and cupric ion, mediated b nitrosonium ion" XP002139628 abstract & J. AM. CHEM. SOC. (1984), 106(11), 3374-6 , --- -/--	1-12

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search 11 August 2000	Date of mailing of the international search report 24.08.00
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Douschan, K

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

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Inte ional Application No
PCT/NL 00/00117

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BOBBITT J M ET AL: "ORGANIC NITROSINIUM SALTS AS OXIDANTS IN ORGANIC CHEMISTRY" HETEROCYCLES,XX,XX, vol. 27, no. 2, 1 January 1988 (1988-01-01), pages 509-533, XP000609709 ISSN: 0385-5414 see especially Table 1 the whole document	1-12
X	NOOY DE A E J ET AL: "HIGHLY SELECTIVE TEMPO MEDIATED OXIDATION OF PRIMARY ALCOHOL GROUPSIN POLYSACCHARIDES" RECUEIL DES TRAVAUX CHIMIQUES DES PAYS-BAS,NL,ELSEVIER SCIENCE PUBLISHERS. AMSTERDAM, vol. 113, no. 3, 1 March 1994 (1994-03-01), pages 165-166, XP000560836 ISSN: 0165-0513 the whole document	1-18
X	NOOY DE A E J ET AL: "ON THE USE OF STABLE ORGANIC NITROXYL RADICALS FOR THE OXIDATION OFPRIMARY AND SECONDARY ALCOHOLS" SYNTHESIS,DE,GEORG THIEME VERLAG. STUTTGART, 1 October 1996 (1996-10-01), pages 1153-1174, XP002072173 ISSN: 0039-7881 the whole document	1-12
X,P	WO 99 23117 A (VALTON TEKNILLINEN TUTKIMUSKESKUS) 14 May 1999 (1999-05-14) the whole document	1-12
X	US 3 632 802 A (J. N. BEMILLER ET AL.) 4 January 1972 (1972-01-04) claims 1,8; example 5	13-18
X	US 5 747 658 A (INSTITUT VOOR AGROTECHNOLOGISCH ONDERZOEK) 5 May 1998 (1998-05-05) claims 10,11; example 1	13-18
A	EP 0 124 439 A (INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE) 7 November 1984 (1984-11-07) the whole document	13-18

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 00/00117

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9923117 A	14-05-1999	FI 974139 A	05-05-1999
		AU 1035199 A	24-05-1999
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		JP 9500414 T	14-01-1997
		WO 9512619 A	11-05-1995
EP 0124439 A	07-11-1984	FR 2545101 A	02-11-1984
		DE 3461938 D	12-02-1987
		JP 59205949 A	21-11-1984
		US 4672034 A	09-06-1987

フロントページの続き

(51)Int.Cl.^{7}	ＦＩ	テーマコード（参考）
// Ｃ０７Ｈ　7/033	Ｃ０７Ｈ　7/033	

(81)指定国　AP(GH,GM,KE,LS,MW,SD,SL,SZ,TZ,UG,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE),OA(BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG),AE,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,NO,NZ,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,US,UZ,VN,YU,ZA,ZW

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Ｆターム(参考)　4B064 AE49 AF12 AF17 AH07 CA21 CB11 CB13 CC03 CD01 CD12
4C057 AA02 AA03 AA19 BB02 EE03
4C090 AA01 AA04 BA06 BA21 BA37 BA41 BA42 BA46 CA34 CA43